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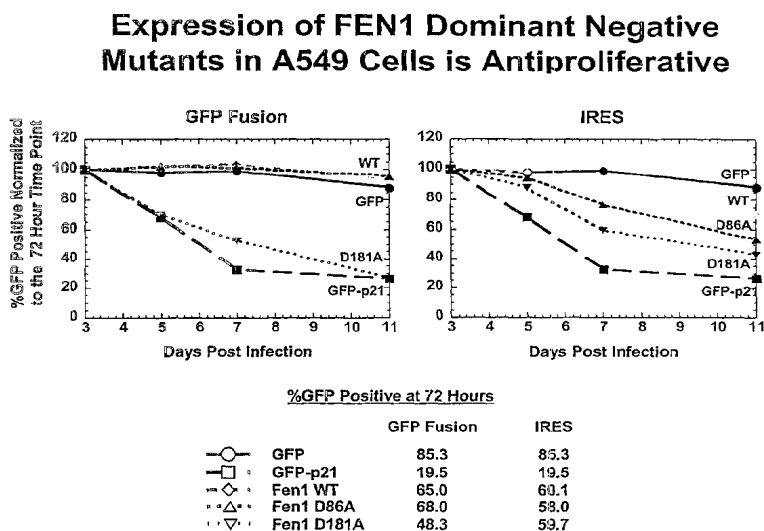
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[Continued on next page]

(54) Title: MODULATORS OF CELLULAR PROLIFERATION



(57) Abstract: The present invention relates to regulation of cellular proliferation. More particularly, the present invention is directed to nucleic acids encoding protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), which are involved in modulation of cell cycle arrest.

The invention further relates to methods for identifying and using agents, including small molecule chemical compositions, antibodies, peptides, cyclic peptides, nucleic acids, RNAi, antisense nucleic acids, and ribozymes, that modulate cell cycle arrest via modulation of protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), as well as to the use of expression profiles and compositions in diagnosis and therapy related to cell cycle regulation and modulation of cellular proliferation, e.g., for treatment of cancer and other diseases of cellular proliferation.



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Modulators of Cellular Proliferation

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of provisional U.S. Application No. 60/395,443,
5 filed July 12, 2002, which is herein incorporated by reference for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] Not applicable.

FIELD OF THE INVENTION

[0003] The present invention relates to regulation of cellular proliferation. More particularly, the present invention is directed to nucleic acids encoding protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine
15 kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine
20 threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), which are involved in modulation of cell cycle arrest. The invention further relates to methods for identifying and using agents, including small molecule chemical compositions, antibodies, peptides, cyclic peptides, nucleic acids, RNAi, antisense nucleic acids, and ribozymes, that modulate cell cycle arrest via modulation
25 of protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7),
30 cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase

(NKIAMRE), or histone acetylase (HBO1), as well as to the use of expression profiles and compositions in diagnosis and therapy related to cell cycle regulation and modulation of cellular proliferation, e.g., for treatment of cancer and other diseases of cellular proliferation.

BACKGROUND OF THE INVENTION

5 [0004] Cell cycle regulation plays a critical role in neoplastic disease, as well as disease caused by non-cancerous, pathologically proliferating cells. Normal cell proliferation is tightly regulated by the activation and deactivation of a series of proteins that constitute the cell cycle machinery. The expression and activity of components of the cell cycle can be altered during the development of a variety of human disease such as cancer, cardiovascular
10 disease, psoriasis, where aberrant proliferation contributes to the pathology of the illness. There are genetic screens to isolate important components for cell cycle regulation using different organisms such as yeast, worms, flies, etc. However, involvement of a protein in cell cycle regulation in a model system is not always indicative of its role in cancer and other proliferative disease. Thus, there is a need to establish screening for understanding human
15 diseases caused by disruption of cell cycle regulation. Identifying proteins, their ligands and substrates, and downstream signal transduction pathways involved in cell cycle regulation and neoplasia in humans is important for developing therapeutic regents to treat cancer and other proliferative diseases.

BRIEF SUMMARY OF THE INVENTION

20 [0005] The present invention therefore provides nucleic acids encoding protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1),
apurinic/aprimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase
25 (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase
(NKIAMRE), or histone acetylase (HBO1), which are involved in modulation of cell cycle arrest in tumor cells and other pathologically proliferating cells. The invention therefore
30 provides methods of screening for compounds, e.g., small organic molecules, antibodies, peptides, cyclic peptides, nucleic acids, antisense molecules, RNAi, and ribozymes, that are capable of modulating cellular proliferation and/or cell cycle regulation, e.g., either inhibiting cellular proliferation, or activating apoptosis. Therapeutic and diagnostic methods and

reagents are also provided. Modulators of protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) are therefore useful in treatment of cancer and other proliferative diseases.

[0006] One embodiment of the present invention provides a method for identifying a compound that modulates cell cycle arrest. A cell comprising a protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or fragment thereof is contacted with the compound. The protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or fragment thereof may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36. The chemical or phenotypic effect of the compound upon the cell comprising the protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP

transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or fragment thereof is determined, thereby identifying a compound that modulates cell cycle arrest. The chemical or phenotypic effect may be determined by measuring enzymatic activity of the protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide. The chemical or phenotypic effect may be determined by measuring cell cycle arrest. The cell cycle arrest may be measured by assaying DNA synthesis or fluorescent marker level. DNA synthesis may be measured by ³H thymidine incorporation, BrdU incorporation, or Hoescht staining. The fluorescent marker may be a cell tracker dye or green fluorescent protein. Modulation may be activation of cell cycle arrest or activation of cancer cell cycle arrest. The host cell may be a cancer cell. The cancer cell may be a breast, prostate, colon, or lung cancer cell. The cancer cell may be a transformed cell line, such as, for example, PC3, H1299, MDA-MB-231, MCF7, A549, or HeLa. The cancer cell may be p53 null, p53 mutant, or p53 wild-type. The polypeptide may be recombinant. The polypeptide may be encoded by a nucleic acid comprising a sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, or 35. The compound may be an antibody, an antisense molecule, a small organic molecule, a peptide, a circular peptide, or an siRNA molecule.

[0007] Another embodiment of the invention provides a method for identifying a compound that modulates cell cycle arrest. The compound is contacted with a protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase

(PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or fragment thereof. The protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or a fragment thereof may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoded by a polypeptide comprising an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36. The physical effect of the compound upon the protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide is determined. The chemical or phenotypic effect of the compound upon a cell comprising a protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2 or CK2 α), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide or fragment thereof is determined, thereby identifying a compound that modulates cell cycle arrest.

[0008] Yet another embodiment of the invention provides a method of modulating cell cycle arrest in a subject. A therapeutically effective amount of a compound identified according to one of the methods described above is administered to the subject. The subject may be a human. The subject may have cancer. The compound may inhibit cancer cell proliferation.

[0009] Even another embodiment of the invention provides a method of modulating cell cycle arrest in a subject. A therapeutically effective amount of a protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide is administered to the subject. The protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36.

[0010] A further embodiment of the invention provides a method of modulating cell cycle arrest in a subject. A therapeutically effective amount of a nucleic acid encoding a protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine

threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1) polypeptide is administered to the subject. The protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET),
 5 flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/aprimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase
 10 (NKIAMRE), or histone acetylase (HBO1) polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36.

[0011] The invention also provides specific siRNA molecules for inhibition of expression
 15 of cell cycle genes. In one embodiment, the invention provides a CK2-specific siRNA molecule comprising the sequence AACATTGAATTAGATCCACGT. The CK2-specific siRNA molecule can be from 21 to 30 nucleotide base pairs in length. In one aspect, the CK2-specific siRNA molecule has the sequence AACATTGAATTAGATCCACGT and its complement as active portion. The CK2-specific siRNA molecules can be used in a method
 20 of inhibiting expression of a CK2 gene in a cell, by contacting the cell with the method comprising contacting the cell with a CK2-specific siRNA molecule from 21 to 30 nucleotide base pairs in length that includes the sequence AACATTGAATTAGATCCACGT.

[0012] In another embodiment, the invention provides a PIM1-specific siRNA molecule comprising the sequence AAAACTCCGAGTGAAGTGGTC. The PIM1-specific siRNA
 25 molecule can be from 21 to 30 nucleotide base pairs in length. In one aspect, the PIM1-specific siRNA molecule has the sequence AAAACTCCGAGTGAAGTGGTC and its complement as active portion. The PIM1-specific siRNA molecules can be used in a method of inhibiting expression of a PIM1 gene in a cell, by contacting the cell with the method comprising contacting the cell with a PIM1-specific siRNA molecule from 21 to 30
 30 nucleotide base pairs in length that includes the sequence AAAACTCCGAGTGAAGTGGTC.

[0013] In another embodiment, the invention provides a Hbo1-specific siRNA molecule comprising the sequence AACTGAGCAAGTGGTTGATTT. The Hbo1-specific siRNA molecule can be from 21 to 30 nucleotide base pairs in length. In one aspect, the Hbo1-specific siRNA molecule has the sequence AACTGAGCAAGTGGTTGATTT and its complement as active portion. The Hbo1-specific siRNA molecules can be used in a method of inhibiting expression of a Hbo1 gene in a cell, by contacting the cell with the method comprising contacting the cell with a Hbo1-specific siRNA molecule from 21 to 30 nucleotide base pairs in length that includes the sequence AACTGAGCAAGTGGTTGATTT.

[0014] Other embodiments and advantages of the present invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 provides a nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of human PKC- ζ .

[0016] Figure 2 provides a nucleotide (SEQ ID NO:3) and an amino acid (SEQ ID NO:4) sequence of human PLC- β 1.

[0017] Figure 3 provides a nucleotide (SEQ ID NO:5) and an amino acid (SEQ ID NO:6) sequence of human FAK.

[0018] Figure 4 provides a nucleotide (SEQ ID NO:7) and an amino acid (SEQ ID NO:8) sequence of human FAK2.

[0019] Figure 5 provides a nucleotide (SEQ ID NO:9) and an amino acid (SEQ ID NO:10) sequence of human CK2.

[0020] Figure 6 provides a nucleotide (SEQ ID NO:11) and an amino acid (SEQ ID NO:12) sequence of human cMET.

[0021] Figure 7 provides a nucleotide (SEQ ID NO:13) and an amino acid (SEQ ID NO:14) sequence of human FEN1.

[0022] Figure 8 provides a nucleotide (SEQ ID NO:15) and an amino acid (SEQ ID NO:16) sequence of human REV1.

[0023] Figure 9 provides a nucleotide (SEQ ID NO:17) and an amino acid (SEQ ID NO:18) sequence of human APE1.

[0024] Figure 10 provides a nucleotide (SEQ ID NO:19) and an amino acid (SEQ ID NO:20) sequence of human CDK3.

[0025] Figure 11 provides a nucleotide (SEQ ID NO:21) and an amino acid (SEQ ID NO:22) sequence of human PIM1.

5 [0026] Figure 12 provides a nucleotide (SEQ ID NO:23) and an amino acid (SEQ ID NO:24) sequence of human CDC7L1.

[0027] Figure 13 provides a nucleotide (SEQ ID NO:25) and an amino acid (SEQ ID NO:26) sequence of human CDK7.

10 [0028] Figure 14 provides a nucleotide (SEQ ID NO:27) and an amino acid (SEQ ID NO:28) sequence of human CNK.

[0029] Figure 15 provides a nucleotide (SEQ ID NO:29) and an amino acid (SEQ ID NO:30) sequence of human PRL-3.

[0030] Figure 16 provides a nucleotide (SEQ ID NO:31) and an amino acid (SEQ ID NO:32) sequence of human STK2 (NEK4).

15 [0031] Figure 17 provides a nucleotide (SEQ ID NO:33) and an amino acid (SEQ ID NO:34) sequence of human NKIAMRE.

[0032] Figure 18 provides a nucleotide (SEQ ID NO:35) and an amino acid (SEQ ID NO:36) sequence of human HBO1.

20 [0033] Figure 19 provides a table summarizing genes that may be involved in the modulation of cell proliferation.

[0034] Figure 20 illustrates inhibition of proliferation of A549 cells by expression of wild-type GFP-CDC7LI and mutant GFP-CDC7LI.

[0035] Figure 21 illustrates inhibition of proliferation of A549 cells by expression of wild-type CNK and mutant GFP-CNK.

25 [0036] Figure 22 illustrates inhibition of proliferation of A549 cells and Hela cells by expression of wild-type and mutant STK2.

[0037] Figure 23 provides amino acid sequences for dominant negative mutants of CDC7L1.

[0038] Figure 24 provides amino acid sequences for dominant negative mutants of CNK.

[0039] Figure 25 provides amino acid sequences for dominant negative mutants of STK2.

[0040] Figure 26 provides an alternative view of the amino acid sequences for dominant negative mutants of CDC7L1.

5 [0041] Figure 27 provides Taqman analysis (*i.e.*, real time PCR) of Cdc7L mRNA expression using RNA from tumor cell lines and primary human cell lines. Cdc7L mRNA levels were normalized to GAPDH mRNA levels.

[0042] Figure 28 provides analysis of CDC7L mRNA levels in matched cancerous and normal tissue from patients with lung carcinoma. Each matched pair represents a different
10 patient.

[0043] Figure 29 provides analysis of CDC7L mRNA in matched cancerous and normal tissue from patients with colon carcinoma. Each matched pair represents a different patient.

[0044] Figure 30 provides Taqman analysis (*i.e.*, real time PCR) of CNK mRNA expression using RNA from tumor cell lines and primary human cell lines. CNK mRNA
15 levels were normalized to GAPDH mRNA levels.

[0045] Figure 31 demonstrates that GST-CNK produced in *E.coli* has kinase activity against p53 and MBP. GST-CNK also exhibits autophosphorylation activity.

[0046] Figure 32 depicts the structure of STK2 long (STK2L) and short (STK2S) forms and their expression levels in human tissues.

20 [0047] Figure 33 provides Taqman analysis (*i.e.*, real time PCR) of STK2 mRNA expression using RNA from tumor cell lines and primary human cell lines. STK2 mRNA levels were normalized to GAPDH mRNA levels.

[0048] Figure 34 demonstrates that GFP-STK2S expression is antiproliferative when measured using the cell tracker assay.

25 [0049] Figure 35 demonstrates that GFP-STK2L expression is antiproliferative in A549 and HeLa cells.

[0050] Figure 36 demonstrates that GFP-STK2L expression is antiproliferative when measured using the cell tracker assay.

[0051] Figure 37 demonstrates that IRES-STK2L expression is antiproliferative in A549 and HeLa cells.

[0052] Figure 38 demonstrates that expression of IRES Hbo1 E508Q is antiproliferative in A549 cells.

5 [0053] Figure 39 demonstrates that no significant differences in proliferation are observed between Hbo1 WT and mutant proteins when expressed in H1299 cells.

[0054] Figure 40 demonstrates that expression of Hbo1 mtant E508Q is antiproliferative in HeLa cells.

10 [0055] Figure 41 depicts analysis of proliferation in sorted cells that express wild type or mutant Hbo1 proteins.

[0056] Figure 42 demonstrates that expression of HBO1 mutant E508Q is antiproliferative in sorted A549 cells.

[0057] Figure 43 demonstrates that expression of HBO1 mutant E508Q is antiproliferative in sorted HeLa cells.

15 [0058] Figure 44 demonstrates that expression of HBO1-specific siRNA reduces Hbo1 mRNA levels and has an antiproliferative effect on A549 cells.

[0059] Figure 45 demonstrates that HBO1-specific siRNA reduces Hbo1 mRNA levels and has an antiproliferative effect on 1299 cells.

20 [0060] Figure 46 provides Taqman analysis (*i.e.*, real time PCR) of PIM1 mRNA expression using RNA from tumor cell lines and primary human cell lines. PIM1 mRNA levels were normalized to 18S RNA levels.

[0061] Figure 47 provides Taqman analysis (*i.e.*, real time PCR) of PIM1 mRNA levels in matched cancerous and normal tissue from patients with breast carcinoma. Each matched pair represents a different patient. PIM1 mRNA levels were normalized to 18S RNA levels.

25 [0062] Figure 48 provides Taqman analysis (*i.e.*, real time PCR) of PIM1 mRNA levels in matched cancerous and normal tissue from patients with lung carcinoma. Each matched pair represents a different patient. PIM1 mRNA levels were normalized to 18S RNA levels.

[0063] Figure 49 demonstrates that expression of PIM1 wild type, but not mutant protein, is antiproliferative in A549 cells.

[0064] Figure 50 demonstrates that expression of GFP-PIM1 wild type is antiproliferative in H1299 cells. The figure also demonstrates that expression of both IRES PIM1 wild type and mutant is antiproliferative in H1299 cells.

[0065] Figure 51 demonstrates that expression of PIM1-specific siRNA reduces PIM1 mRNA levels and has an antiproliferative effect on A549 cells.

[0066] Figure 52 demonstrates that expression of PIM1-specific siRNA reduces PIM1 mRNA levels and has an antiproliferative effect on HeLa cells.

[0067] Figure 53 demonstrates that expression of PIM1-specific siRNA reduces PIM1 mRNA levels and has an antiproliferative effect on H1299 cells.

[0068] Figure 54 demonstrates that expression of PIM1-specific siRNA reduces PIM1 mRNA levels and has an antiproliferative effect on primary HUVEC cells.

[0069] Figure 55 demonstrates that expression of APE1 wild type and mutant proteins is not antiproliferative in A549 cells.

[0070] Figure 56 demonstrates that expression of APE1 wild type and mutant proteins is not antiproliferative in H1299 cells.

[0071] Figure 57 demonstrates that expression of APE1 wild type and APE1 D210A mutant proteins is antiproliferative in primary HMEC cells.

[0072] Figure 58 demonstrates that expression of the Ape1 D210A mutant sensitizes A549 cells to methyl methanesulfonate treatment.

[0073] Figure 59 demonstrates that wild type Ape1 and the Ape1 C65A mutant are protective when expressed in A549 cells treated with bleomycin.

[0074] Figure 60 demonstrates that wild type Ape1 and the Ape1 C65A mutant are protective when expressed in HeLa cells or H1299 cells treated with bleomycin.

[0075] Figure 61 provides Taqman analysis (*i.e.*, real time PCR) of CK2 α mRNA expression using RNA from tumor cell lines and primary cell lines. CK2 α mRNA levels were normalized to 18S RNA levels.

[0076] Figure 62 provides the sequence of dominant negative mutants of CK2 α .

[0077] Figure 63 demonstrates that expression of CK2 α -specific siRNA reduces CK2 α mRNA levels and has an antiproliferative effect on H1299 cells.

[0078] Figure 64 provides Taqman analysis (*i.e.*, real time PCR) of NKIAMRE expression using RNA from tumor cell lines and primary cell lines. NKIAMRE mRNA levels were normalized to 18S RNA levels.

[0079] Figure 65 provides the sequence of dominant negative mutants of NKIAMRE.

5 [0080] Figure 66 provides the sequence of dominant negative mutants of FEN1.

[0081] Figure 67 demonstrates that expression of FEN1 dominant negative mutants in A549 cells is antiproliferative.

[0082] Figure 68 demonstrates that expression of FEN1 dominant negative mutants in H1299 cells is antiproliferative.

10 [0083] Figure 69 provides the sequence of dominant negative mutants of CDK3.

[0084] Figure 70 demonstrates that expression of GFP-CDK3 wild type and CDK3 mutant proteins appears to have no antiproliferative effect in A549 cells. The figure also demonstrates that expression of both IRES CDK3 wild type and CDK3 mutant proteins appears to have no antiproliferative effect in A549 cells.

15 [0085] Figure 71 demonstrates that expression of GFP-CDK3 wild type and CDK3 mutant proteins appears to have no antiproliferative effect in H1299 cells. The figure also demonstrates that expression of both IRES CDK3 wild type and CDK3 mutant proteins appears to have no antiproliferative effect in H1299 cells.

[0086] Figure 72 provides the sequence of dominant negative mutants of HBO1.

20 [0087] Figure 73 provides the sequence of dominant negative mutants of PIM1.

[0088] Figure 74 demonstrates that expression of GFP-NKIAMRE wild type and NKIAMRE mutant proteins appears to have no antiproliferative effect in either A549 cells or H1299 cells.

DETAILED DESCRIPTION OF THE INVENTION

25 INTRODUCTION

[0089] PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, and HBO1 encode proteins involved in modulation of the cell cycle in cancer cells.

[0090] As described below, the present inventors identified PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, and HBO1 as modulators of the cell cycle in immunoprecipitation assays or yeast 2 hybrid assays.

5 [0091] PKC- ζ encodes an atypical isoform of protein kinase C, *i.e.*, an isoform that is not activated by phorbol esters or diacylglycerols (*see, e.g.*, Donson *et al. J. Neuro-Onc.*, 47:109 (2000)). PKC- ζ activates several signaling pathways, mediates multiple cellular functions, and plays a role in the proliferation of fibroblast cells, endothelial cells, smooth muscle cells, human glioblastoma cells, and astrocytoma cells (*see, e.g.*, Guizzetti and Costa, *Biochem.*
10 *Pharmacol.*, 60:1457 (2000); Donson *et al.*, 2000). PKC- ζ also plays a role in the activation of p70 S6 kinase which modulates the progression through the G₁ phase of the cell cycle (*see*, Guizzetti, 2000). Assays known to those of skill in the art can be used to identify modulators of PKC- ζ (*see, e.g.*, *J. Biol. Chem.*, 276:3543; *J. Biol. Chem.*, 272:31130; *J. Biol. Chem.*, 270:15884; *J. Biol. Chem.*, 273:26277; *J. Biol. Chem.*, 272:16578; *Mol. Cell. Biol.*, 19:2180).
15 For example, IRS-1, nucleoli, heterogeneous ribonucleoprotein A1, Sp1, Sendai virus phosphoprotein, and IKK β may be used as substrates in assays to identify modulators of PKC- ζ (*see, e.g.*, *J. Biol. Chem.*, 276:3543; *J. Biol. Chem.*, 272:31130; *J. Biol. Chem.*, 270:15884; *J. Biol. Chem.*, 273:26277; *J. Biol. Chem.*, 272:16578; *Mol. Cell. Biol.*, 19:2180).

[0092] PLC- β 1 encodes a phosphoinositide-specific phospholipase C. The PLC- β 1 isoform
20 is the predominant nuclear phospholipase C in multiple cell types, including erythroleukemia cells, osteosarcoma cells, pheochromocytoma cells, and glioma cells (*see, e.g.*, Cocco *et al.*, *Advan. Enzyme Regul.*, 39:287 (1999)). PLC- β 1 has been shown to be responsible for nuclear inositol lipid metabolism in multiple cell types (*see, e.g.*, Avazeri, *et al.*, *Mol. Biol. Cell*, 11:4369 (2000)). Overexpression of PLC- β 1 in human colon cancer cells suppresses
25 tumor cell growth, but induces increased cell aggregation and increased expression and release of carcinoembryonic antigen molecule (*see, e.g.*, Nomoto *et al.*, *Jpn. J. Canc. Res.*, 89:1257 (1998)). PLC- β 1 has been reported to be essential for IGF-1 induced mitogenesis (*see*, Cocco *et al.*, 1999). Phospholipase C activity assays known to those of skill in the art can be used to identify modulators of PLC- β 1 (*see, e.g.*, Nomoto *et al.*, 1998; *Physiol. Rev.*,
30 80:1291 (2000); *Biochemistry*, 36:848; *Eur. J. Biochem.*, 213:339). For example, phosphoinositide may be used as a substrate in assays to identify modulators of PLC- β 1 (*see, e.g.*, Nomoto *et al.*, 1998; and *Physiol. Rev.*, 80:1291 (2000); *Biochemistry*, 36:848; *Eur. J. Biochem.*, 213:339). Additional assays to identify modulators of PLC- β 1 are described in,

e.g., 109 Mark Dolittle and Karen Reue, *Methods in Molecular Biology: Lipase and Phospholipase Protocols* (1998)

[0093] FAK encodes a cytoplasmic tyrosine kinase that plays a role in regulation of cell cycle progression (*see, e.g.*, MacPhee *et al.*, *Lab. Invest.*, 81(11):1469 (2001) and Zhao *et al.*, 5 *Mol. Biol. Cell*, 12:4066 (2001)). Specifically, FAK regulates cell cycle progression by increasing cyclin D1 expression and/or decreasing expression of the CDK inhibitor p21 (*see*, Zhao *et al.*, 2001). High levels of FAK have been linked to tumor invasiveness and metastasis (*see, e.g.*, Fresu *et al.*, *Biochem. J.*, 358:407 (2001)). Tyrosine kinase assays known to those of skill in the art can be used to identify modulators of FAK (*see, e.g.*, 10 *Bioessays*, 19:137; *Mol. Biol. Cell*, 10:2507 (1999)). For example, p130Cas and paxillin may be used as a substrate to identify modulators of FAK (*see, e.g.*, *Bioessays*, 19:137; *Mol. Biol. Cell*, 10:2507 (1999)).

[0094] FAK2 encodes a calcium dependent tyrosine kinase that localizes to sites of cell-to-cell contact and participates in cellular signal transduction (*see, e.g.*, Sasaki *et al.*, *J. Bio.* 15 *Chem.*, 270(6):21206 (1995) and Li *et al.*, *J. Biol. Chem.*, 273(16):9361 (1998)). Tyrosine kinase assays known to those of skill in the art can be used to identify modulators of FAK2 (*see, e.g.*, Sasaki *et al.*, 1995). For example, p130Cas and paxillin may be used as substrates in assays to identify modulators of FAK2.

[0095] CK2 or CK2 α encodes an ubiquitous serine threonine protein kinase that is required 20 for the G₂/M transition and checkpoint control stages of the cell cycle (*see, e.g.*, Messenger *et al.*, *J. Biol. Chem.* 277:23054 (2002), Sayed *et al.*, *Oncogene* 20(48):6994 (2001), and Escargueil *et al.* *J. Biol. Chem.* 275(44):34710 (2000)). In particular, CK2 is required for the phosphorylation of topoisomerase 1 during the G₂/M transition of the cell cycle (*see*, Messenger *et al.*, 2002). CK2 is overexpressed in tumors and leukemic cells (*see*, Messenger 25 *et al.*, 2002). CK2 works with p53 in spindle checkpoint arrest to maintain increase cyclin B/cdc2 kinase activity (*see*, Sayed *et al.*, 2001). Serine threonine protein kinase assays known to those of skill in the art can be used in assays to identify modulators of CK2 (*see, e.g.*, Messenger *et al.*, 2002 and *J. Biol. Chem.*, 274(41):29260).

[0096] cMET encodes a tyrosine kinase that is expressed in numerous tissues and plays a 30 role in the generation and spread of tumors of the stomach, rectum, lung, pancreas, breast, and bile duct (*see, e.g.*, Jeffers *et al.*, *Proc. Nat'l. Acad. Sci. USA* 94:11445 (1997) and Ramirez *et al.*, *Endocrinology* 53:635 (2000)). More specifically, cMET plays a role in

angiogenesis, cell motility, cell growth, cell invasion, and morphogenic differentiation (*see, Jeffers et al., 1997*). In particular, cMET overexpression is associated with a high risk of metastasis and recurrence of papillary thyroid carcinoma (*see, Ramirez et al., 2000*).

Tyrosine kinase assays known to those of skill in the art can be used in assays to identify modulators of cMET (*see, Jeffers et al., 1997*). For example dCMP, Grb2, Gab can be used as substrates in assays to identify modulators of cMET.

[0097] FEN1 encodes a structure specific endonuclease that cleaves substrates with unannealed 5' tails (*see, e.g., Warbrick et al., J. Pathol. 186:319 (1998)*). FEN1 has high specificity of binding/activity toward 5' flap structures, *i.e.*, dsDNA with a displaced 5' strand (*see, e.g., Warbrick et al., 1998 and Tom et al., J. Biol. Chem. 275(14):10498 (2000)*). FEN1 also exhibits a 5' to 3' exonucleolytic activity. FEN1 levels are low in non-cycling cells and are induced as the cells enter the cell cycle (*see, Warbrick et al., 1998*). FEN1 assays known to those of skill in the art can be used to identify modulators of FEN1 (*see, Tom et al., 2000 and EMBO J., 13(5):1235 (1994)*). For example, 5' DNA flap structures can be used as substrates in assays to identify modulators of FEN1 (*see, e.g., EMBO J., 13(5):1235 (1994)*).

[0098] REV1 encodes a 1251 amino acid dCMP transferase that functions in the Pol ζ mutagenesis pathway (*see, e.g., Lui et al., Nuc. Acids. Res. 27(22):4468 (1999) and Zhang et al., Nuc. Acids Res. 30(7):1630 (2002)*). REV1 has been implicated in UV induced mutagenesis repair and is postulated to play a role in UV damage tolerance (*see, e.g., Murakomo, J. Biol. Chem., 276(38):35644 (2001)*). dCMP transferase assays known to those of skill in the art can be used to identify modulators of REV1 (*see, Zhang et al., 2002 and J. Biol. Chem., 276(18):15051*). For example, dCMP, 5'-end 32P-labeled oligonucleotide primer 5'-CACTGACTGTATG-3' annealed to an oligonucleotide template, 5'-CTCGTCAGCATCTTCAUCATACAGTCAGTG-3' treated with uracil-DNA glycosylase may be used as substrates in assays to identify modulators of REV1 (*see, e.g., J. Biol. Chem., 276(18):15051*).

[0099] APE1 encodes an apyrimidinic endonuclease that plays a role in short patch repair and long patch repair of ionizing radiation and alkylating agent induced damage in DNA (*see, e.g., Tom et al., J. Biol. Chem., 276(52):48781 (2001), Izumi, Carcinogenesis, 21(7):1329 (2000), and Bobola et al., Clin. Cancer Res. 7(11):3510 (2001)*). APE1 has also plays a role the cellular response to oxidative stress, regulation of transcription factors, cell

cycle control, and apoptosis (*see*, Bobola *et al.*, 2001). Assays known to those of skill in the art can be used to identify modulators of APE1 (*see*, Tom *et al.*, 2001 and Bobola *et al.*, 2001; *Nucleic Acids Res.*, 5(4):1413 (1978); *Biochimie*, 64(8-9):603 (1982); *Mutat. Res.*, 460(3-4):211 (2000)). For example, oligonucleotide duplexes containing an
5 apurinic/apyrimidinic sites may be used as a substrate in assays to identify modulators of APE1.

[0100] CDK3 encodes a cyclin dependent kinase that regulates entry into S phase. (*see*, *e.g.*, Braun *et al.*, *Oncogene*, 17(7):2259 (1998)). Specifically, CDK3 has been described as a positive G₁ phase regulator that enhances the G₁/S transition (*see*, Braun *et al.*, *Oncogene*,
10 1998). Overexpression of CDK2 and CDK3 together has been show to elevate c-myc induced apoptosis (*see, e.g.*, Braun *et al.*, *DNA Cell Biol.*, 17(9):789 (1998)). A dominant negative mutant of CDK3 suppresses apoptosis and overexpression of CDK3 circumvents the anti-apoptotic effect of bcl-2 (*see, e.g.*, Meikrantz and Schlegel, *J. Biol. Chem.*, 271(17):10205 (1996)). Assays known to those of skill in the art can be used to identify
15 modulators of CDK3 (*see, e.g.*, *Eur. J. Biochem.*, 268:6076 (2001)). For example, pRb, histone H1, and P701K3-1 (the C-terminal domain of RNA Pol I) may used as substrates in assays to identify modulators of CDK3 (*see, e.g.*, *Eur. J. Biochem.*, 268:6076 (2001)).

[0101] PIM1 encodes two cytoplasmic serine threonine kinases generated by an alternate translation initiation (*see, e.g.*, Mochizuki *et al.*, *Oncogene* 15:1471 (1997) and Shirogane *et al.*, *Immunity* 11:709 (1999)). PIM1 plays a role in cellular transformation and inhibits
20 apoptosis (*see, e.g.*, Mochizuki *et al.*, 1997). Specifically, PIM1 cooperates with c-myc to promote cell proliferation through the G₁ to S transition and to prevent apoptosis (Shirogane *et al.*, 1999). PIM1 has been implicated in T cell lymphoma, *i.e.*, it has been shown that PIM1 cooperates with the oncoprotein E2a-Pbx1 to facilitate thymic lymphagenesis (*see, e.g.*,
25 Feldman *et al.*, *Oncogene* 15(22):2735 (1997)). Assays known to those of skill in the art can be used to identify modulators of PIM1 (*see, e.g.*, *J. Biol. Chem.*, 266(21):14018). For example, histone H1 may be used as a substrate in assays to identify modulators of PIM1 (*see, e.g.*, *J. Biol. Chem.*, 266(21):14018).

[0102] CDC7L1 encodes a 574 amino acid serine threonine kinase (*see, e.g.*, Masai and
30 Arai, *J. Cell Physiol.*, 190(3):287 (2002), Masai *et al.*, *J. Biol. Chem.*, 275(37):29042 (2000), and Johnston *et al.*, *Prog. Cell Cycle Res.*, 4:61(2002)). CDC7L1 binds the activator for S phase kinase (ASK) to form a complex that is present at high levels during S phase and

decreased levels during G₁ phase. Assays known to those of skill in the art can be used to identify modulators of CDC7L1 (*see, e.g., Masai et al., 2000; Johnston et al., 2000; and Proc. Natl. Acad. Sci. USA, 94:14320 (1997)*). For example, histone H1 may be used as a substrate in assays to identify modulators of CDC7L1 (*see, e.g., Proc. Natl. Acad. Sci. USA, 94:14320 (1997)*). Alternatively, Mcm2 may be used as a substrate in assays to identify modulators of CDC7L1 (*see, e.g., Takeda et al., Mol. Biol. Cell, 12:1257 (2001)*). Conditional muCDC7-deficient embryonic cell lines and transgenic CDC7 knockout mice have been generated (*see, e.g., EMBO J. 21:L2168 (2002)*). The cell lines undergo S phase arrest and the knockout mouse is embryonic lethal.

[0103] CDK7 encodes a cyclin dependent kinase that is postulated to play a role in cell cycle regulation (*see, e.g., Nishiwaki et al., Mol. Cell Biol., 20(20):7726 (2000), Acevedo-Duncan et al., Cell. Prolif. 35(1):23 (2002), and Bregman et al., Front. Biosci., 5:D244 (2000)*). CDK7 is the kinase component of the transcription factor complex TFIIH and has been shown to contribute to the ability of p16^{INK4A} to induce cell cycle arrest (*see, Nishiwaki et al., 2002*). Assays known to those of skill in the art can be used to identify modulators of CDK7 (*see, e.g., Mol. Cell. Biol., 21:88 (2001)*). For example, CDK2 and the C-terminal domain of RNA Pol II can be used as substrates in assays to identify modulators of CDK7.

[0104] CNK is also known as PRK (Proliferation related kinase) and encodes a cytokine inducible serine threonine kinase (*see, e.g., Li et al., J. Biol. Chem. 271 (32):19402 (1996), Dai et al., Genes Chromosomes Cancer, 27(3):332 (2000), and Ouyang et al., Oncogene, 18(44):6029 (1999)*). CNK is a member of the polo family of kinases which have been implicated in cell division (*see, Li et al., 1996*). CNK expression is downregulated in lung cancer and in head and neck cancer (*see, Li et al., 1996 and Dai et al., 2000*). Assays known to those of skill in the art can be used to identify modulators of CNK (*see, e.g., J. Biol. Chem., 272:28646*). For example, CDC25, p53, and casein can be used as substrates in assays to identify modulators of CNK (*see, e.g., J. Biol. Chem., 272:28646*).

[0105] PRL-3 encodes a 22 kDa potentially prenylated protein tyrosine phosphatase (*see, e.g., Zeng et al., Biochem. Biophys. Res. Commun. 244(2):421 (1998), Saha et al., Science, 294(5545):1343 (2001), and Bradbury, Lancet 358(9289):1245 (2001)*). PRL-3 is localized to the cytoplasmic membrane when prenylated at its carboxy terminus, and to the nucleus when it is not prenylated (*see, Saha et al., 2001*). PRL-3 is expressed at low levels in normal colorectal epithelial cells, at intermediate levels in malignant stage I or II cancers, and at high

levels in colorectal metastases (*see*, Saha *et al.*, 2001). Assays known to those of skill in the art can be used to identify modulators of PRL-3.

[0106] STK2 is also known as NEK4 and encodes a serine threonine kinase (*see, e.g.*, Chen *et al.*, *Gene*, 234(1):127 (1999), Hayashi *et al.*, *Biochem. Biophys. Res. Commun.*, 264(2):449 (1999) and Levedakou *et al.*, *Oncogene* 9(7):1977 (1994). STK2 (NEK4) has been localized to chromosome 3p21.1 and is a member of the NIMA family of kinases which are G₂/M regulators of the cell cycle. Assays known to those of skill in the art can be used to identify modulators of STK2 (NEK4) (*see*, Hayashi *et al.*, 1999; *Biochem. Biophys. Res. Commun.*, 264(2):449 (1999); *J. Biol. Chem.* 269:6603 (1994)). For example, the polypeptide FRXT can be used as a substrate in assays to modulate STK2 function.

[0107] NKIAMRE encodes the human homologue to the mitogen-activated protein kinase-/cyclin-dependent kinase-related protein kinase NKIATRE (*see, e.g.*, Midermer *et al.*, *Cancer Res.*, 59(16):4069 (1999)). NKIAMRE localizes to chromosome band 5q31 and is deleted in samples from leukemia patients (*see, e.g.*, Midermer *et al.*, 1999). Assays known to those of skill in the art can be used to identify modulators of NKIAMRE.

[0108] HBO1 encodes a member of the MYST family of histone acetyltransferases (*see, e.g.*, Iizuka and Stillman, *J. Biol. Chem.*, 274(33):23027 (1999), Sterner and Berger, *Microbiol. Mol. Biol. Rev.*, 64(2):435 (2000), and Burke *et al.*, *J. Biol. Chem.* 276(18):15397 (2001)). HBO1 binds to ORC (origin recognition complex) to form a complex that plays a role in the initiation of replication (*see*, Sterner and Berger, 2000). Assays known to those of skill in the art can be used to identify modulators of HBO1 (*see*, Iizuka and Stillman, 1999 and *J. Bio. Chem.*, 274(33):23027 (1999)). For example, histone H3 and histone H4 can be used as substrates in assays to identify modulators of HBO1 (*see, e.g.*, *J. Bio. Chem.*, 274(33):23027 (1999)).

[0109] Thus, protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), flap structure specific endonuclease 1 (FEN1), REV1 dCMP transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent serine threonine kinase

(NKIAMRE), and histone acetylase (HBO1) can conveniently be used to identify agents that modulate the cell cycle.

[0110] PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, and HBO1 therefore represent drug targets for compounds that suppress or activate cellular proliferation in tumor cells, or cause cell cycle arrest, cause release from cell cycle arrest, activate apoptosis, increase sensitivity to chemotherapeutic (adjuvant) reagents, and decrease toxicity of chemotherapeutic reagents. Agents identified in these assays, including small organic molecules, peptides, cyclic peptides, nucleic acids, antibodies, antisense nucleic acids, RNAi, and ribozymes, that modulate cell cycle regulation and cellular proliferation via modulation of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, can be used to treat diseases related to cellular proliferation, such as cancer. In particular, inhibitors of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 are useful for inhibition of cancer and tumor cell growth. PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators can also be used to modulate the sensitivity of cells to chemotherapeutic agents, such as bleomycin, etoposide, taxol, and other agents known to those of skill in the art. PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators can also be used to decrease toxicity of such chemotherapeutic reagents.

[0111] In one embodiment, enzymatic assays, including kinase or autophosphorylation assays, lipase assays, nuclease assays, transferase assays, phosphatase assays, and acetylase assays using PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be used to identify modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 activity, or to identify proteins that bind to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, e.g., PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 substrates. Full length wild type PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1,

CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, mutant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be used in these assays.

[0112] Such modulators are useful for treating cancers, such as melanoma, breast, ovarian, lung, gastrointestinal and colon, prostate, and leukemia and lymphomas, e.g., multiple myeloma. In addition, such modulators are useful for treating noncancerous disease states caused by pathologically proliferating cells such as thyroid hyperplasia (Grave's disease), psoriasis, benign prostatic hypertrophy, neurofibromas, atherosclerosis, restenosis, and other vasoproliferative disease.

10 DEFINITIONS

[0113] By "disorder associated with cellular proliferation" or "disease associated with cellular proliferation" herein is meant a disease state which is marked by either an excess or a deficit of cellular proliferation or apoptosis. Such disorders associated with increased cellular proliferation include, but are not limited to, cancer and non-cancerous pathological proliferation.

[0114] The terms "PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1" or a nucleic acid encoding "PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1" refer to nucleic acids and polypeptide polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 60% amino acid sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of over a region of at least about 25, 50, 100, 200, 500, 1000, or more amino acids, to an amino acid sequence encoded by a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acid (for a human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acid sequence, *see, e.g.*, Figures 1-18, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 or Accession number NM_002744, NM_015192, L05186, L49207, NM_001895, J02958, NM_004111, AF206019, X66133, NM_001258, M16750, NM_003503, NM_001799, NM_004073, NM_007079, XM_003216, AF130372, or

NM_007067 or amino acid sequence of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein (for a human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein sequence, *see, e.g.*, Figures 1-18, SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or Accession number AAA36488, NP_056007, AAA35819, Q14289, NP_001886, AAA59591, NP_004102, AAF18986, S34422, NP_001249, AAA60089, NP_003494, NP_001790, NP_004064, NP_009010, XP_003216, AAF36509, and NP_008998; (2) bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to an anti-sense strand corresponding to a nucleic acid sequence encoding a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, and conservatively modified variants thereof; (4) have a nucleic acid sequence that has greater than about 95%, preferably greater than about 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, or more nucleotides, to a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acid or a nucleic acid encoding the enzymatic domain. Preferably the enzymatic domain has greater than 96%, 97%, 98%, or 99% amino acid identity to the human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 enzymatic domain of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 35, or 36. A polynucleotide or polypeptide sequence is typically from a mammal including, but not limited to, primate, e.g., human; rodent, e.g., rat, mouse, hamster; cow, pig, horse, sheep, or any mammal. The nucleic acids and proteins of the invention include both naturally occurring or recombinant molecules.

[0115] The phrase “functional effects” in the context of assays for testing compounds that modulate activity of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein includes the determination of a parameter that is indirectly or directly under the influence of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1,

CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, e.g., a phenotypic or chemical effect, such as the ability to increase or decrease cellular proliferation, apoptosis, cell cycle arrest, or enzymatic activity, or e.g., a physical effect such as ligand binding or inhibition of ligand binding. A functional effect therefore includes ligand binding activity, the ability of cells to proliferate, apoptosis, and enzyme activity. “Functional effects” include *in vitro*, *in vivo*, and *ex vivo* activities.

[0116] By “determining the functional effect” is meant assaying for a compound that increases or decreases a parameter that is indirectly or directly under the influence of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, e.g., measuring physical and chemical or phenotypic effects. Such functional effects can be measured by any means known to those skilled in the art, e.g., changes in spectroscopic characteristics (e.g., fluorescence, absorbance, refractive index); hydrodynamic (e.g., shape); chromatographic; or solubility properties for the protein; measuring inducible markers or transcriptional activation of the protein; measuring binding activity or binding assays, e.g. binding to antibodies; measuring changes in ligand or substrate binding activity; measuring cellular proliferation; measuring cell morphology, e.g., spindle formation or chromosome formation; measuring phosphorylated proteins such as histone H3 using antibodies; measuring apoptosis; measuring cell surface marker expression; measurement of changes in protein levels for PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1-associated sequences; measurement of RNA stability; identification of downstream or reporter gene expression (CAT, luciferase, β -gal, GFP and the like), e.g., via chemiluminescence, fluorescence, colorimetric reactions, antibody binding, and inducible markers.

[0117] “Inhibitors”, “activators”, and “modulators” of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 polynucleotide and polypeptide sequences are used to refer to activating, inhibitory, or modulating molecules identified using *in vitro* and *in vivo* assays of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 polynucleotide and polypeptide sequences. Inhibitors are compounds that, e.g., bind to, partially or totally block activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity or expression of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3,

PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins, e.g., antagonists. "Activators" are compounds that increase, open, activate, facilitate, enhance activation, sensitize, agonize, or up regulate PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein activity, e.g., agonists. Inhibitors, activators, or modulators also include genetically modified versions of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins, e.g., versions with altered activity, as well as naturally occurring and synthetic ligands, antagonists, agonists, antibodies, peptides, cyclic peptides, nucleic acids, siRNA molecules, antisense molecules, ribozymes, small chemical molecules and the like. Such assays for inhibitors and activators include, e.g., expressing PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein *in vitro*, in cells, or cell membranes, applying putative modulator compounds, and then determining the functional effects on activity, as described above.

[0118] Samples or assays comprising PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative protein activity value of 100%. Inhibition of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 is achieved when the activity value relative to the control is about 80%, preferably 50%, more preferably 25-0%. Activation of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 is achieved when the activity value relative to the control (untreated with activators) is 110%, more preferably 150%, more preferably 200-500% (i.e., two to five fold higher relative to the control), more preferably 1000-3000% higher.

[0119] The term "test compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, e.g., protein, oligopeptide (e.g., from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide,

oligonucleotide, etc., to be tested for the capacity to directly or indirectly modulation tumor cell proliferation. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, e.g., targeting compounds, rescue
5 compounds, dimerization compounds, stabilizing compounds, addressable compounds, and other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound (called a “lead compound”) with some desirable property or activity, e.g., inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput
10 screening (HTS) methods are employed for such an analysis.

[0120] A “small organic molecule” refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 daltons and less than about 2500 daltons, preferably less than about 2000 daltons, preferably between about 100 to about 1000 daltons, more preferably between about 200 to about 500 daltons.

15 [0121] An “siRNA” refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA expressed in the same cell as the gene or target gene. “siRNA” thus refers to the double stranded RNA formed by the complementary strands. siRNA molecule and RNAi molecule are used interchangeably herein. The complementary portions of the siRNA
20 that hybridize to form the double stranded molecule typically have substantial or complete identity. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA. In another embodiment, a “randomized siRNA” refers to a nucleic acid that forms a double stranded siRNA, wherein the sequence of the siRNA is randomized. The sequence of the siRNA can
25 correspond to the full length target gene, or a subsequence thereof. Typically, the siRNA is at least about 15-50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferably about 15-30 nucleotides in length, preferably about 20-30 nucleotides in length, preferably about 21-30 nucleotides in length, or about 20-25 or about
30 24-29 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[0122] “Biological sample” include sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. Such samples include blood, sputum, tissue, cultured cells, e.g., primary cultures, explants, and transformed cells, stool, urine, etc. A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0123] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (e.g., nucleotide sequence SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 or amino acid sequence SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36), when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. Such sequences are then said to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0124] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0125] A “comparison window”, as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may

be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981),
5 by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see*,
10 e.g., *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

[0126] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1997) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the
15 parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when
20 aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the
25 parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-
30 scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the

BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

5 [0127] “Nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the
10 reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0128] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions)
15 and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The
20 term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0129] A particular nucleic acid sequence also implicitly encompasses “splice variants.” Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. “Splice variants,” as the name suggests, are
25 products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction,
30 including recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, *et al.*, *J. Biol. Chem.* 273(52):35095-35101 (1998).

[0130] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0131] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0132] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0133] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes

every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0134] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0135] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g., Creighton, Proteins* (1984)).

[0136] Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, *see, e.g., Alberts et al., Molecular Biology of the Cell* (3rd ed., 1994) and Cantor and Schimmel, *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (1980).

“Primary structure” refers to the amino acid sequence of a particular peptide. “Secondary structure” refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, e.g., enzymatic domains, extracellular domains, transmembrane domains, pore domains, and cytoplasmic tail domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 15 to 350 amino acids long. Exemplary domains include domains with enzymatic activity, e.g., a kinase domain. Typical domains are made up of sections of lesser organization such as stretches of β -sheet and α -helices. “Tertiary structure” refers to the complete three

dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

[0137] A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

[0138] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0139] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0140] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal

melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium).

5 Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and
10 0.1% SDS at 65°C.

[0141] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize
15 under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency. Additional
20 guidelines for determining hybridization parameters are provided in numerous reference, e.g., and *Current Protocols in Molecular Biology*, ed. Ausubel, *et al.*

[0142] For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is
25 typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1 - 2 min. Protocols and guidelines for low and high stringency amplification reactions are
30 provided, e.g., in Innis *et al.* (1990) *PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc. N.Y.).

[0143] “Antibody” refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding.

[0144] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0145] Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H - C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990))

[0146] For preparation of antibodies, *e.g.*, recombinant, monoclonal, or polyclonal antibodies, many technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan,

Current Protocols in Immunology (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, *Immunology* (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks *et al.*, *Bio/Technology* 10:779-783 (1992); Lonberg *et al.*, *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991); and Suresh *et al.*, *Methods in Enzymology* 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (see, e.g., U.S. Patent No. 4,676,980, WO 91/00360; WO 92/200373; and EP 03089).

[0147] Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988) and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric

antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0148] A “chimeric antibody” is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[0149] In one embodiment, the antibody is conjugated to an “effector” moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein.

[0150] The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, polymorphic variants, alleles, orthologs, and conservatively modified variants, or splice variants, or portions thereof, can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically

immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

- 5 [0151] By “therapeutically effective dose” herein is meant a dose that produces effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g., Lieberman, Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

10 ASSAYS FOR PROTEINS THAT MODULATE CELLULAR PROLIFERATION

- [0152] High throughput functional genomics assays can be used to identify modulators of cellular proliferation. Such assays can monitor changes in cell surface marker expression, proliferation and differentiation, and apoptosis, using either cell lines or primary cells. Typically, the cells are contacted with a cDNA or a random peptide library (encoded by
15 nucleic acids). In one embodiment, the peptides are cyclic or circular. The cDNA library can comprise sense, antisense, full length, and truncated cDNAs. The peptide library is encoded by nucleic acids. The effect of the cDNA or peptide library on the phenotype of cellular proliferation is then monitored, using an assay as described above. The effect of the cDNA or peptide can be validated and distinguished from somatic mutations, using, e.g., regulatable
20 expression of the nucleic acid such as expression from a tetracycline promoter. cDNAs and nucleic acids encoding peptides can be rescued using techniques known to those of skill in the art, e.g., using a sequence tag.

- [0153] Proteins interacting with the peptide or with the protein encoded by the cDNA (e.g., PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1,
25 CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1) can be isolated using a yeast two-hybrid system, mammalian two hybrid system, immunoprecipitation or affinity chromatography of complexed proteins followed by mass spectrometry, or phage display screen, etc. Targets so identified can be further used as bait in these assays to identify additional members of the cellular proliferation pathway, which members are also targets for
30 drug development (*see, e.g., Fields et al., Nature* 340:245 (1989); Vasavada et al., *Proc. Nat'l Acad. Sci. USA* 88:10686 (1991); Fearon et al., *Proc. Nat'l Acad. Sci. USA* 89:7958 (1992); Dang et al., *Mol. Cell. Biol.* 11:954 (1991); Chien et al., *Proc. Nat'l Acad. Sci. USA*

9578 (1991); and U.S. Patent Nos. 5,283,173, 5,667,973, 5,468,614, 5,525,490, and 5,637,463).

[0154] Suitable cell lines include A549, HeLa, Colo205, H1299, MCF7, MDA-MB-231, PC3, HMEC, PrEC. Cell surface markers can be assayed using fluorescently labeled
 5 antibodies and FACS. Cell proliferation can be measured using ³H-thymidine incorporation, cell count by dye inclusion, MTT assay, BrdU incorporation, Cell Tracker assay. Apoptosis can be measured using dye inclusion, or by assaying for DNA laddering, increases in intracellular calcium, or caspase activation. Growth factor production can be measured using an immunoassay such as ELISA.

10 [0155] cDNA libraries are made from any suitable source. Libraries encoding random peptides are made according to techniques well known to those of skill in the art (*see, e.g.,* U.S. Patent No. 6,153,380, 6,114,111, and 6,180,343). Any suitable vector can be used for the cDNA and peptide libraries, including, *e.g.,* retroviral vectors.

15 **ISOLATION OF NUCLEIC ACIDS ENCODING PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 FAMILY MEMBERS**

[0156] This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al., Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and*
 20 *Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al., eds., 1994*)).

[0157] PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acids, polymorphic variants, orthologs, and alleles that are substantially identical to an amino acid
 25 sequence encoded by SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 can be isolated using PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acid probes and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone PKC- ζ , PLC- β 1, FAK,
 30 FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, polymorphic variants, orthologs, and alleles by detecting expressed homologs immunologically with antisera or purified antibodies made

against human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 or portions thereof.

[0158] To make a cDNA library, one should choose a source that is rich in PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 RNA. The mRNA is then made into cDNA using reverse transcriptase, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. Methods for making and screening cDNA libraries are well known (*see, e.g., Gubler & Hoffman, Gene 25:263-269 (1983); Sambrook et al., supra; Ausubel et al., supra*).

[0159] For a genomic library, the DNA is extracted from the tissue and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged *in vitro*. Recombinant phage are analyzed by plaque hybridization as described in Benton & Davis, *Science* 196:180-182 (1977). Colony hybridization is carried out as generally described in Grunstein *et al., Proc. Natl. Acad. Sci. USA., 72:3961-3965 (1975)*.

[0160] An alternative method of isolating PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 nucleic acid and its orthologs, alleles, mutants, polymorphic variants, and conservatively modified variants combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (*see U.S. Patents 4,683,195 and 4,683,202; PCR Protocols: A Guide to Methods and Applications (Innis et al., eds, 1990)*). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid

sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes.

5 Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

[0161] Gene expression of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can also be analyzed by techniques known in the art, e.g., reverse transcription and
10 amplification of mRNA, isolation of total RNA or poly A⁺ RNA, northern blotting, dot blotting, *in situ* hybridization, RNase protection, high density polynucleotide array technology, e.g., and the like.

[0162] Nucleic acids encoding PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1
15 protein can be used with high density oligonucleotide array technology (e.g., GeneChipTM) to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, orthologs, alleles, conservatively modified variants, and polymorphic variants in this invention. In the case where the homologs being identified are linked to modulation of cellular proliferation,
20 they can be used with GeneChipTM as a diagnostic tool in detecting the disease in a biological sample, *see, e.g.*, Gunthand *et al.*, *AIDS Res. Hum. Retroviruses* 14: 869-876 (1998); Kozal *et al.*, *Nat. Med.* 2:753-759 (1996); Matson *et al.*, *Anal. Biochem.* 224:110-106 (1995); Lockhart *et al.*, *Nat. Biotechnol.* 14:1675-1680 (1996); Gingeras *et al.*, *Genome Res.* 8:435-448 (1998); Hacia *et al.*, *Nucleic Acids Res.* 26:3865-3866 (1998).

25 [0163] The gene for PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 is typically cloned into intermediate vectors before transformation into prokaryotic or eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, e.g., plasmids, or shuttle vectors.

30 **EXPRESSION IN PROKARYOTES AND EUKARYOTES**

[0164] To obtain high level expression of a cloned gene, such as those cDNAs encoding PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1,

CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, one typically subclones PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, e.g., in Sambrook *et al.*, and Ausubel *et al. supra*. Bacterial expression systems for expressing the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein are available in, e.g., *E. coli*, *Bacillus sp.*, and *Salmonella* (Palva *et al.*, *Gene* 22:229-235 (1983); Mosbach *et al.*, *Nature* 302:543-545 (1983). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available. In one preferred embodiment, retroviral expression systems are used in the present invention.

[0165] Selection of the promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

[0166] In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operably linked to the nucleic acid sequence encoding PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. Additional elements of the cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

[0167] In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient

termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

[0168] The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in

5 eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as MBP, GST, and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, e.g., c-myc. Sequence tags may be included in an expression cassette for nucleic acid rescue. Markers such as fluorescent proteins, green or
10 red fluorescent protein, β -gal, CAT, and the like can be included in the vectors as markers for vector transduction.

[0169] Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, retroviral vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic
15 vectors include pMSG, pAV009/A⁺, pMTO10/A⁺, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

20 [0170] Expression of proteins from eukaryotic vectors can be also be regulated using inducible promoters. With inducible promoters, expression levels are tied to the concentration of inducing agents, such as tetracycline or ecdysone, by the incorporation of response elements for these agents into the promoter. Generally, high level expression is obtained from inducible promoters only in the presence of the inducing agent; basal
25 expression levels are minimal.

[0171] In one embodiment, the vectors of the invention have a regulatable promoter, e.g., tet-regulated systems and the RU-486 system (*see, e.g.,* Gossen & Bujard, *Proc. Nat'l Acad. Sci. USA* 89:5547 (1992); Oligino *et al.*, *Gene Ther.* 5:491-496 (1998); Wang *et al.*, *Gene Ther.* 4:432-441 (1997); Neering *et al.*, *Blood* 88:1147-1155 (1996); and Rendahl *et al.*, *Nat. Biotechnol.* 16:757-761 (1998)). These impart small molecule control on the expression of
30 the candidate target nucleic acids. This beneficial feature can be used to determine that a desired phenotype is caused by a transfected cDNA rather than a somatic mutation.

[0172] Some expression systems have markers that provide gene amplification such as thymidine kinase and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable, such as using a baculovirus vector in insect cells, with a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 encoding sequence under the direction of the polyhedrin promoter or other strong baculovirus promoters.

[0173] The elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are preferably chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

[0174] Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, which are then purified using standard techniques (*see, e.g., Colley et al., J. Biol. Chem.* 264:17619-17622 (1989); *Guide to Protein Purification, in Methods in Enzymology*, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (*see, e.g., Morrison, J. Bact.* 132:349-351 (1977); Clark-Curtiss & Curtiss, *Methods in Enzymology* 101:347-362 (Wu et al., eds, 1983)).

[0175] Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, biolistics, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g., Sambrook et al., supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1.

[0176] After the expression vector is introduced into the cells, the transfected cells are cultured under conditions favoring expression of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, which is recovered from the culture using standard techniques
5 identified below.

PURIFICATION OF PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 POLYPEPTIDES

[0177] Either naturally occurring or recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be purified for use in functional assays. Naturally occurring PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be purified, e.g., from human tissue. Recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be purified
10
15 from any suitable expression system.

[0178] The PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (*see, e.g., Scopes, Protein Purification: Principles and Practice* (1982); U.S. Patent No. 4,673,641; Ausubel *et al.*, *supra*; and Sambrook *et al.*, *supra*).
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[0179] A number of procedures can be employed when recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. With the appropriate ligand or substrate, e.g., antiphospho S/T antibodies or anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibodies, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1
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protein can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein could be purified using immunoaffinity columns. Recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be purified from any suitable source, include yeast, insect, bacterial, and mammalian cells.

A. Purification of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 from recombinant bacteria

[0180] Recombinant proteins are expressed by transformed bacteria in large amounts, typically after promoter induction; but expression can be constitutive. Promoter induction with IPTG is one example of an inducible promoter system. Bacteria are grown according to standard procedures in the art. Fresh or frozen bacteria cells are used for isolation of protein.

[0181] Proteins expressed in bacteria may form insoluble aggregates ("inclusion bodies"). Several protocols are suitable for purification of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein inclusion bodies. For example, purification of inclusion bodies typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells, e.g., by incubation in a buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria are apparent to those of skill in the art (*see, e.g., Sambrook et al., supra; Ausubel et al., supra*).

[0182] If necessary, the inclusion bodies are solubilized, and the lysed cell suspension is typically centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents which are capable of solubilizing aggregate-forming proteins, for example SDS (sodium dodecyl sulfate), 70% formic acid, are inappropriate for use in this

procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known to those skilled in the art. Human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins are separated from other bacterial proteins by standard separation techniques, e.g., with Ni-NTA agarose resin.

[0183] Alternatively, it is possible to purify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein from bacteria periplasm. After lysis of the bacteria, when the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to skill in the art. To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM MgSO₄ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.

B. Standard protein separation techniques for purifying PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins

Solubility fractionation

[0184] Often as an initial step, particularly if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium

sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

Size differential filtration

[0185] The molecular weight of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins can be used to isolate it from proteins of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

Column chromatography

[0186] The PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins can also be separated from other proteins on the basis of its size, net surface charge, hydrophobicity, and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

ASSAYS FOR MODULATORS OF PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 PROTEIN

A. Assays

5 [0187] Modulation of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, and corresponding modulation of cellular, e.g., tumor cell, proliferation, can be assessed using a variety of *in vitro* and *in vivo* assays, including cell-based models. Such assays can be used to test for inhibitors and activators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, and, consequently, inhibitors and activators of cellular proliferation, including modulators of chemotherapeutic sensitivity and toxicity. Such modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein are useful for treating disorders related to pathological cell proliferation, e.g., cancer. Modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein are tested using either recombinant or naturally occurring PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, preferably human PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1.

[0188] Preferably, the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein will have the sequence as encoded by SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or a conservatively modified variant thereof. Alternatively, the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein of the assay will be derived from a eukaryote and include an amino acid subsequence having substantial amino acid sequence identity to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36. Generally, the amino acid sequence identity will be at least 60%, preferably at least 65%, 70%, 75%, 80%, 85%, or 90%, most preferably at least 95%.

[0189] Measurement of cellular proliferation modulation with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or a cell expressing PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, either recombinant or naturally occurring, can be performed using a variety of assays, *in vitro*, *in vivo*, and *ex vivo*, as described herein. A suitable physical, chemical or phenotypic change that affects activity, e.g., enzymatic activity such as kinase activity, cell proliferation, or ligand binding can be used to assess the influence of a test compound on the polypeptide of this invention. When the functional effects are determined using intact cells or animals, one can also measure a variety of effects, such as, ligand binding, kinase activity, transcriptional changes to both known and uncharacterized genetic markers (e.g., northern blots), changes in cell metabolism, changes related to cellular proliferation, cell surface marker expression, DNA synthesis, marker and dye dilution assays (e.g., GFP and cell tracker assays), contact inhibition, tumor growth in nude mice, etc.

In vitro assays

[0190] Assays to identify compounds with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulating activity can be performed *in vitro*. Such assays can use full length PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or a variant thereof (*see, e.g.*, SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36), or a mutant thereof, or a fragment of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, such as a kinase domain. Purified recombinant or naturally occurring PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be used in the *in vitro* methods of the invention. In addition to purified PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, the recombinant or naturally occurring PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be part of a cellular lysate or a cell membrane. As described below, the binding assay can be either solid state or soluble.

Preferably, the protein or membrane is bound to a solid support, either covalently or non-covalently. Often, the *in vitro* assays of the invention are substrate or ligand binding or affinity assays, either non-competitive or competitive. Other *in vitro* assays include measuring changes in spectroscopic (e.g., fluorescence, absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties for the protein. Other *in vitro* assays include enzymatic activity assays, such as phosphorylation or autophosphorylation assays.

[0191] In one embodiment, a high throughput binding assay is performed in which the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or a fragment thereof is contacted with a potential modulator and incubated for a suitable amount of time. In one embodiment, the potential modulator is bound to a solid support, and the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is added. In another embodiment, the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is bound to a solid support. A wide variety of modulators can be used, as described below, including small organic molecules, peptides, antibodies, and PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 ligand analogs. A wide variety of assays can be used to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1-modulator binding, including labeled protein-protein binding assays, electrophoretic mobility shifts, immunoassays, enzymatic assays such as kinase assays, and the like. In some cases, the binding of the candidate modulator is determined through the use of competitive binding assays, where interference with binding of a known ligand or substrate is measured in the presence of a potential modulator. Either the modulator or the known ligand or substrate is bound first, and then the competitor is added. After the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is washed, interference with binding, either of the potential modulator or of the known ligand or substrate, is determined. Often, either the potential modulator or the known ligand or substrate is labeled.

Cell-based *in vivo* assays

[0192] In another embodiment, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is expressed in a cell, and functional, e.g., physical and chemical or phenotypic, changes are assayed to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 and modulators of cellular proliferation, e.g., tumor cell proliferation. Cells expressing PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins can also be used in binding assays and enzymatic assays. Any suitable functional effect can be measured, as described herein. For example, cellular morphology (e.g., cell volume, nuclear volume, cell perimeter, and nuclear perimeter), ligand binding, kinase activity, apoptosis, cell surface marker expression, cellular proliferation, GFP positivity and dye dilution assays (e.g., cell tracker assays with dyes that bind to cell membranes), DNA synthesis assays (e.g., ^3H -thymidine and fluorescent DNA-binding dyes such as BrdU or Hoescht dye with FACS analysis), are all suitable assays to identify potential modulators using a cell based system. Suitable cells for such cell based assays include both primary cancer or tumor cells and cell lines, as described herein, e.g., A549 (lung), MCF7 (breast, p53 wild-type), H1299 (lung, p53 null), Hela (cervical), PC3 (prostate, p53 mutant), MDA-MB-231 (breast, p53 wild-type). Cancer cell lines can be p53 mutant, p53 null, or express wild type p53. The PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be naturally occurring or recombinant. Also, fragments of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 or chimeric PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins with enzymatic activity can be used in cell based assays.

[0193] Cellular PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 polypeptide levels can be determined by measuring the level of protein or mRNA. The level of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or proteins related to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7,

CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 are measured using immunoassays such as western blotting, ELISA and the like with an antibody that selectively binds to the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 polypeptide or a fragment thereof. For measurement of mRNA, amplification, e.g., using PCR, LCR, or hybridization assays, e.g., northern hybridization, RNase protection, dot blotting, are preferred. The level of protein or mRNA is detected using directly or indirectly labeled detection agents, e.g., fluorescently or radioactively labeled nucleic acids, radioactively or enzymatically labeled antibodies, and the like, as described herein.

[0194] Alternatively, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 expression can be measured using a reporter gene system. Such a system can be devised using a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein promoter operably linked to a reporter gene such as chloramphenicol acetyltransferase, firefly luciferase, bacterial luciferase, β -galactosidase and alkaline phosphatase. Furthermore, the protein of interest can be used as an indirect reporter via attachment to a second reporter such as red or green fluorescent protein (*see, e.g.,* Mistili & Spector, *Nature Biotechnology* 15:961-964 (1997)). The reporter construct is typically transfected into a cell. After treatment with a potential modulator, the amount of reporter gene transcription, translation, or activity is measured according to standard techniques known to those of skill in the art.

Animal models

[0195] Animal models of cellular proliferation also find use in screening for modulators of cellular proliferation. Similarly, transgenic animal technology including gene knockout technology, for example as a result of homologous recombination with an appropriate gene targeting vector, or gene overexpression, will result in the absence or increased expression of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1. The same technology can also be applied to make knock-out cells. When desired, tissue-specific expression or knockout of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein may be necessary. Transgenic animals generated by such methods find use as animal models of

cellular proliferation and are additionally useful in screening for modulators of cellular proliferation.

[0196] Knock-out cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into an endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting an endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 with a mutated version of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene, or by mutating an endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, e.g., by exposure to carcinogens.

[0197] A DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (*see, e.g., Capecchi et al., Science* 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan *et al., Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988) and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed., IRL Press, Washington, D.C., (1987).

Exemplary assays

Enzymatic activity assays-- *in vitro* or cell based

[0198] In one embodiment, enzymatic assays using PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be used to identify modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 activity, or to identify proteins that bind to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, e.g., PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4),

NKIAMRE, or HBO1 substrates. Full length wild type PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, mutant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, or the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 enzymatic domain can be used in these assays. Such assays can be performed *in vitro*, using recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 or cellular lysates comprising endogenous or recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, or can be cell-based.

Soft agar growth or colony formation in suspension

- [0199] Normal cells require a solid substrate to attach and grow. When the cells are transformed, they lose this phenotype and grow detached from the substrate. For example, transformed cells can grow in stirred suspension culture or suspended in semi-solid media, such as semi-solid or soft agar. The transformed cells, when transfected with tumor suppressor genes, regenerate normal phenotype and require a solid substrate to attach and grow.
- [0200] Soft agar growth or colony formation in suspension assays can be used to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. Typically, transformed host cells (e.g., cells that grow on soft agar) are used in this assay. For example, RKO or HCT116 cell lines can be used. Techniques for soft agar growth or colony formation in suspension assays are described in Freshney, *Culture of Animal Cells a Manual of Basic Technique*, 3rd ed., Wiley-Liss, New York (1994), herein incorporated by reference. *See also*, the methods section of Garkavtsev *et al.* (1996), *supra*, herein incorporated by reference.

Contact inhibition and density limitation of growth

- [0201] Normal cells typically grow in a flat and organized pattern in a petri dish until they touch other cells. When the cells touch one another, they are contact inhibited and stop growing. When cells are transformed, however, the cells are not contact inhibited and continue to grow to high densities in disorganized foci. Thus, the transformed cells grow to a

higher saturation density than normal cells. This can be detected morphologically by the formation of a disoriented monolayer of cells or rounded cells in foci within the regular pattern of normal surrounding cells. Alternatively, labeling index with [³H]-thymidine at saturation density can be used to measure density limitation of growth. *See* Freshney (1994),
 5 *supra*. The transformed cells, when contacted with cellular proliferation modulators, regenerate a normal phenotype and become contact inhibited and would grow to a lower density.

[0202] Contact inhibition and density limitation of growth assays can be used to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1,
 10 CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators which are capable of inhibiting abnormal proliferation and transformation in host cells. Typically, transformed host cells (e.g., cells that are not contact inhibited) are used in this assay. For example, RKO or HCT116 cell lines can be used. In this assay, labeling index with [³H]-thymidine at saturation density is a preferred method of measuring density limitation of growth.

15 Transformed host cells are contacted with a potential PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulator and are grown for 24 hours at saturation density in non-limiting medium conditions. The percentage of cells labeling with [³H]-thymidine is determined autoradiographically. *See*, Freshney (1994), *supra*. The host cells contacted with
 20 a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulator would give rise to a lower labeling index compared to control (e.g., transformed host cells transfected with a vector lacking an insert).

Growth factor or serum dependence

25 [0203] Growth factor or serum dependence can be used as an assay to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. Transformed cells have a lower serum dependence than their normal counterparts (*see, e.g.,* Temin, *J. Natl. Cancer Inst.* 37:167-175 (1966); Eagle *et al.*, *J. Exp. Med.* 131:836-879 (1970)); Freshney, *supra*.

30 This is in part due to release of various growth factors by the transformed cells. When transformed cells are contacted with a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or

HBO1 modulator, the cells would reacquire serum dependence and would release growth factors at a lower level.

Tumor specific markers levels

[0204] Tumor cells release an increased amount of certain factors (hereinafter “tumor specific markers”) than their normal counterparts. For example, plasminogen activator (PA) is released from human glioma at a higher level than from normal brain cells (*see, e.g.,* Gullino, *Angiogenesis, tumor vascularization, and potential interference with tumor growth*. In Mihich (ed.): “Biological Responses in Cancer.” New York, Academic Press, pp. 178-184 (1985)). Similarly, tumor angiogenesis factor (TAF) is released at a higher level in tumor cells than their normal counterparts. *See, e.g.,* Folkman, *Angiogenesis and cancer, Sem Cancer Biol.* (1992)).

[0205] Tumor specific markers can be assayed to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators which decrease the level of release of these markers from host cells. Typically, transformed or tumorigenic host cells are used. Various techniques which measure the release of these factors are described in Freshney (1994), *supra*. Also, *see, Unkless et al., J. Biol. Chem.* 249:4295-4305 (1974); Strickland & Beers, *J. Biol. Chem.* 251:5694-5702 (1976); Whur *et al., Br. J. Cancer* 42:305-312 (1980); Gulino, *Angiogenesis, tumor vascularization, and potential interference with tumor growth*. In Mihich, E. (ed): “Biological Responses in Cancer.” New York, Plenum (1985); Freshney *Anticancer Res.* 5:111-130 (1985).

Invasiveness into Matrigel

[0206] The degree of invasiveness into Matrigel or some other extracellular matrix constituent can be used as an assay to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators which are capable of inhibiting abnormal cell proliferation and tumor growth. Tumor cells exhibit a good correlation between malignancy and invasiveness of cells into Matrigel or some other extracellular matrix constituent. In this assay, tumorigenic cells are typically used as host cells. Therefore, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators can be identified by measuring changes in the level of invasiveness between the host cells before and after the introduction of potential

modulators. If a compound modulates PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, its expression in tumorigenic host cells would affect invasiveness.

[0207] Techniques described in Freshney (1994), *supra*, can be used. Briefly, the level of invasion of host cells can be measured by using filters coated with Matrigel or some other extracellular matrix constituent. Penetration into the gel, or through to the distal side of the filter, is rated as invasiveness, and rated histologically by number of cells and distance moved, or by prelabeling the cells with ^{125}I and counting the radioactivity on the distal side of the filter or bottom of the dish. *See, e.g.*, Freshney (1984), *supra*.

Apoptosis analysis

[0208] Apoptosis analysis can be used as an assay to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. In this assay, cell lines, such as RKO or HCT116, can be used to screen PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. Cells are contacted with a putative PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulator. The cells can be co-transfected with a construct comprising a marker gene, such as a gene that encodes green fluorescent protein, or a cell tracker dye. The apoptotic change can be determined using methods known in the art, such as DAPI staining and TUNEL assay using a fluorescent microscope. For TUNEL assay, commercially available kit can be used (e.g., Fluorescein FragEL DNA Fragmentation Detection Kit (Oncogene Research Products, Cat.# QIA39) + Tetramethyl-rhodamine-5-dUTP (Roche, Cat. # 1534 378)). Cells contacted with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators would exhibit, e.g., an increased apoptosis compared to control.

Cell cycle arrest analysis

[0209] Cell cycle arrest can be used as an assay to identify PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. In this assay, cell lines, such as RKO or HCT116, can be used to screen PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1,

APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. The cells can be co-transfected with a construct comprising a marker gene, such as a gene that encodes green fluorescent protein, or a cell tracker dye. Methods known in the art can be used to measure the degree of cell cycle arrest. For example, a propidium iodide signal can be used as a measure for DNA content to determine cell cycle profiles on a flow cytometer. The percent of the cells in each cell cycle can be calculated. Cells contacted with a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulator would exhibit, *e.g.*, a higher number of cells that are arrested in G₁/G₀ phase, G₁/S phase, S/G₂ phase, G₂/M phase, or M/G₂ phase compared to control.

Tumor growth *in vivo*

[0210] Effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators on cell growth can be tested in transgenic or immune-suppressed mice (*e.g.*, xenograft models).

Knock-out transgenic mice can be made, in which the endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene is disrupted. Such knock-out mice can be used to study effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, *e.g.*, as a cancer model, as a means of assaying *in vivo* for compounds that modulate PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, and to test the effects of restoring a wild-type or mutant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 to a knock-out mice.

[0211] Knock-out cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into the endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting the endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 with a mutated version of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, or by mutating the endogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2,

cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, e.g., by exposure to carcinogens.

[0212] A DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (*see, e.g., Capecchi et al., Science* 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan *et al., Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988) and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed., IRL Press, Washington, D.C., (1987). These knock-out mice can be used as hosts to test the effects of various PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators on cell growth.

[0213] Alternatively, various immune-suppressed or immune-deficient host animals can be used. For example, genetically athymic “nude” mouse (*see, e.g., Giovanella et al., J. Natl. Cancer Inst.* 52:921 (1974)), a SCID mouse, a thymectomized mouse, or an irradiated mouse (*see, e.g., Bradley et al., Br. J. Cancer* 38:263 (1978); Selby *et al., Br. J. Cancer* 41:52 (1980)) can be used as a host for, e.g., xenografts. Transplantable tumor cells (typically about 10^6 cells), such as, for example, human tumor cells, injected into isogenic hosts will produce invasive tumors in a high proportions of cases, while normal cells of similar origin will not. Hosts are treated with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators, e.g., by injection. After a suitable length of time, preferably 4-8 weeks, tumor growth is measured (e.g., by volume or by its two largest dimensions) and compared to the control. Tumors that have statistically significant reduction (using, e.g., Student’s T test) are said to have inhibited growth. Using reduction of tumor size as an assay, PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators which are capable, e.g., of inhibiting abnormal cell proliferation can be identified.

B. Modulators

[0214] The compounds tested as modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be any small organic molecule, or a biological entity, such as a protein, e.g., an antibody or peptide, a sugar, a nucleic acid, e.g., an antisense oligonucleotide or a ribozyme, or a lipid. Alternatively, modulators can be genetically altered versions of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. Typically, test compounds will be small organic molecules, peptides, circular peptides, RNAi, antisense molecules, ribozymes, and lipids.

[0215] Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds that can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs Switzerland) and the like.

[0216] In one preferred embodiment, high throughput screening methods involve providing a combinatorial small organic molecule or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[0217] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of

amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[0218] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al.*, *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.*, PCT Publication No. WO 91/19735), encoded peptides (*e.g.*, PCT Publication No. WO 93/20242), random bio-oligomers (*e.g.*, PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.*, *Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al.*, *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al.*, *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e.g.*, U.S. Patent 5,539,083), antibody libraries (*see, e.g.*, Vaughn *et al.*, *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.*, Liang *et al.*, *Science*, 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.*, benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[0219] Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, N.J.; Asinex, Moscow, RU; Tripos, Inc., St. Louis, MO; ChemStar, Ltd, Moscow, RU; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, etc.).

C. *Solid state and soluble high throughput assays*

[0220] In one embodiment the invention provides soluble assays using a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, or a cell or tissue expressing a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, either naturally occurring or recombinant. In another embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 substrate is attached to a solid phase. Any one of the assays described herein can be adapted for high throughput screening.

[0221] In the high throughput assays of the invention, either soluble or solid state, it is possible to screen up to several thousand different modulators or ligands in a single day. This methodology can be used for PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins *in vitro*, or for cell-based or membrane-based assays comprising a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay many plates per day; assay screens for up to about 6,000, 20,000, 50,000, or more than 100,000 different compounds are possible using the integrated systems of the invention.

[0222] For a solid state reaction, the protein of interest or a fragment thereof, e.g., an extracellular domain, or a cell or membrane comprising the protein of interest or a fragment thereof as part of a fusion protein can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage. A tag for covalent or non-covalent binding can be any of a variety of components. In general, a molecule which binds the tag (a tag

binder) is fixed to a solid support, and the tagged molecule of interest is attached to the solid support by interaction of the tag and the tag binder.

[0223] A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.). Antibodies to molecules with natural binders such as biotin and appropriate tag binders are also widely available; *see*, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).

[0224] Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs. For example, agonists and antagonists of cell membrane receptors (e.g., cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherein family, the integrin family, the selectin family, and the like; *see, e.g.*, Pigott & Power, *The Adhesion Molecule Facts Book I* (1993). Similarly, toxins and venoms, viral epitopes, hormones (e.g., opiates, steroids, etc.), intracellular receptors (e.g. which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[0225] Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[0226] Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly gly sequences of between about 5 and 200

amino acids. Such flexible linkers are known to persons of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

5 [0227] Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl
10 groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. *See, e.g., Merrifield, J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen *et al., J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring,
15 *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al., Science*, 251:767-777 (1991); Sheldon *et al., Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al., Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV
20 radiation, and the like.

IMMUNOLOGICAL DETECTION OF PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 POLYPEPTIDES

[0228] In addition to the detection of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1,
25 REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene and gene expression using nucleic acid hybridization technology, one can also use immunoassays to detect PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1
30 proteins of the invention. Such assays are useful for screening for modulators of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, as well as for therapeutic and diagnostic applications. Immunoassays can be used to qualitatively or quantitatively analyze PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7,

CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

A. Production of antibodies

- 5 [0229] Methods of producing polyclonal and monoclonal antibodies that react specifically with the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins are known to those of skill in the art (*see, e.g.,* Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); and
- 10 Kohler & Milstein, *Nature* 256:495-497 (1975). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g.,* Huse *et al.*, *Science* 246:1275-1281 (1989); Ward *et al.*, *Nature* 341:544-546 (1989)).
- 15 [0230] A number of immunogens comprising portions of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein may be used to produce antibodies specifically reactive with PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. For
- 20 example, recombinant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or an antigenic fragment thereof, can be isolated as described herein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen for the
- 25 production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Naturally occurring protein may also be used either in pure or impure form. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays
- 30 to measure the protein.

[0231] Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (e.g., BALB/C mice) or rabbits is immunized with the protein

using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the beta subunits. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see*, Harlow & Lane, *supra*).

[0232] Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see*, Kohler & Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse, *et al.*, *Science* 246:1275-1281 (1989).

[0233] Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non- PKC- ζ , PLC- $\beta 1$, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μ M, preferably at least about 0.1 μ M or better, and most preferably, 0.01 μ M or better. Antibodies specific only for a particular PKC- ζ , PLC- $\beta 1$, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 ortholog, such as human PKC- ζ , PLC- $\beta 1$, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1, can also be made, by subtracting out other cross-reacting orthologs from a species such as a non-human mammal. In this manner, antibodies that bind only to PKC- ζ , PLC- $\beta 1$, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1,

CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein may be obtained.

[0234] Once the specific antibodies against PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4),

5 NKIAMRE, or HBO1 protein are available, the protein can be detected by a variety of immunoassay methods. In addition, the antibody can be used therapeutically as a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 modulators. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*.

B. Immunological binding assays

[0235] PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein can be detected and/or quantified using any of a number of well recognized immunological binding assays (*see, e.g.*, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed.

20 1991). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to a protein or antigen of choice (in this case the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein or antigenic subsequence thereof). The antibody (e.g., anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1) may be produced by any of a number of means well known to those of skill in the art and as described above.

[0236] Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 or a labeled anti-

PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibody. Alternatively, the labeling agent may be a third moiety, such a secondary antibody, that specifically binds to the antibody/ PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 complex (a secondary antibody is typically specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the label agent. These proteins exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (*see, e.g., Kronval et al., J. Immunol.* 111:1401-1406 (1973); *Akerstrom et al., J. Immunol.* 135:2589-2542 (1985)). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are well known to those skilled in the art.

[0237] Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, optionally from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, antigen, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

20 Non-competitive assay formats

[0238] Immunoassays for detecting PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 in samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In one preferred "sandwich" assay, for example, the anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibodies can be bound directly to a solid substrate on which they are immobilized. These immobilized antibodies then capture PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 present in the test sample. PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 proteins thus immobilized are then bound by a labeling agent, such as a second PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3,

PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second or third antibody is typically modified with a detectable moiety, such as biotin, to which another molecule specifically binds, e.g., streptavidin, to provide a detectable moiety.

Competitive assay formats

[0239] In competitive assays, the amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein present in the sample is measured indirectly by measuring the amount of a known, added (exogenous) PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein displaced (competed away) from an anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibody by the unknown PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein present in a sample. In one competitive assay, a known amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is added to a sample and the sample is then contacted with an antibody that specifically binds to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein. The amount of exogenous PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein bound to the antibody is inversely proportional to the concentration of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein bound to the antibody may be determined either by measuring the amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 present in PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1,

CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein may be
 5 detected by providing a labeled PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 molecule.

[0240] A hapten inhibition assay is another preferred competitive assay. In this assay the known PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1,
 10 CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein is immobilized on a solid substrate. A known amount of anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibody is added to the sample, and the sample is then contacted with the immobilized PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3,
 15 PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1. The amount of anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibody bound to the known immobilized PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 is
 20 inversely proportional to the amount of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein present in the sample. Again, the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or
 25 indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

Cross-reactivity determinations

[0241] Immunoassays in the competitive binding format can also be used for crossreactivity determinations. For example, a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 can be immobilized to a solid support. Proteins (e.g., PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 and homologs) are added to the assay that
 30

compete for binding of the antisera to the immobilized antigen. The ability of the added proteins to compete for binding of the antisera to the immobilized protein is compared to the ability of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein to compete with itself. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the added proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorption with the added considered proteins, e.g., distantly related homologs.

[0242] The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be perhaps an allele or polymorphic variant of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, to the immunogen protein. In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required to inhibit 50% of binding is less than 10 times the amount of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein that is required to inhibit 50% of binding, then the second protein is said to specifically bind to the polyclonal antibodies generated to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 immunogen.

Other assay formats

[0243] Western blot (immunoblot) analysis is used to detect and quantify the presence of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1. The anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3,

PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibodies specifically bind to the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 on the solid support. These antibodies may be directly labeled or alternatively may be subsequently
5 detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 antibodies.

[0244] Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or
10 markers. The released chemicals are then detected according to standard techniques (*see* Monroe *et al.*, *Amer. Clin. Prod. Rev.* 5:34-41 (1986)).

Reduction of non-specific binding

[0245] One of skill in the art will appreciate that it is often desirable to minimize non-specific binding in immunoassays. Particularly, where the assay involves an antigen or
15 antibody immobilized on a solid substrate it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this technique involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used with powdered milk
20 being most preferred.

Labels

[0246] The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable
25 physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (e.g., DYNABEADSTM), fluorescent
30 dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and

others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (e.g., polystyrene, polypropylene, latex, etc.).

[0247] The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[0248] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to another molecules (e.g., streptavidin) molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein, or secondary antibodies that recognize anti- PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1.

[0249] The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidotases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labeling or signal producing systems that may be used, see U.S. Patent No. 4,391,904.

[0250] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Colorimetric or chemiluminescent

labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[0251] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

CELLULAR TRANSFECTION AND GENE THERAPY

[0252] The present invention provides the nucleic acids of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein for the transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for the transfection of target cells and organisms as described below. The nucleic acids are transfected into cells, *ex vivo* or *in vivo*, through the interaction of the vector and the target cell. The nucleic acid, under the control of a promoter, then expresses a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 protein of the present invention, thereby mitigating the effects of absent, partial inactivation, or abnormal expression of a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKIAMRE, or HBO1 gene, particularly as it relates to cellular proliferation. The compositions are administered to a patient in an amount sufficient to elicit a therapeutic response in the patient. An amount adequate to accomplish this is defined as "therapeutically effective dose or amount."

[0253] Such gene therapy procedures have been used to correct acquired and inherited genetic defects, cancer, and other diseases in a number of contexts. The ability to express artificial genes in humans facilitates the prevention and/or cure of many important human diseases, including many diseases which are not amenable to treatment by other therapies (for a review of gene therapy procedures, see Anderson, *Science* 256:808-813 (1992); Nabel & Felgner, *TIBTECH* 11:211-217 (1993); Mitani & Caskey, *TIBTECH* 11:162-166 (1993); Mulligan, *Science* 926-932 (1993); Dillon, *TIBTECH* 11:167-175 (1993); Miller, *Nature* 357:455-460 (1992); Van Brunt, *Biotechnology* 6(10):1149-1154 (1998); Vigne, *Restorative*

Neurology and Neuroscience 8:35-36 (1995); Kremer & Perricaudet, *British Medical Bulletin* 51(1):31-44 (1995); Haddada *et al.*, in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al.*, *Gene Therapy* 1:13-26 (1994)).

PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

5 [0254] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (e.g., nucleic acid, protein, modulatory compounds or transduced cell), as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences*, 17th
10 ed., 1989). Administration can be in any convenient manner, e.g., by injection, oral administration, inhalation, transdermal application, or rectal administration.

[0255] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount
15 of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening
20 agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

25 [0256] The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0257] Formulations suitable for parenteral administration, such as, for example, by
30 intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation

isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of compositions can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[0258] Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

[0259] The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The dose will be determined by the efficacy of the particular vector employed and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, or transduced cell type in a particular patient.

[0260] In determining the effective amount of the vector to be administered in the treatment or prophylaxis of conditions owing to diminished or aberrant expression of the PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 (NEK4), NKLAMRE, or HBO1 protein, the physician evaluates circulating plasma levels of the vector, vector toxicities, progression of the disease, and the production of anti-vector antibodies. In general, the dose equivalent of a naked nucleic acid from a vector is from about 1 μ g to 100 μ g for a typical 70 kilogram patient, and doses of vectors which include a retroviral particle are calculated to yield an equivalent amount of therapeutic nucleic acid.

[0261] For administration, compounds and transduced cells of the present invention can be administered at a rate determined by the LD-50 of the inhibitor, vector, or transduced cell type, and the side-effects of the inhibitor, vector or cell type at various concentrations, as applied to the mass and overall health of the patient. Administration can be accomplished via single or divided doses.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Identification of Genes That Modulate Cell Proliferation Using Immunoprecipitation Assays

[0262] PKC ζ , PLC β 1, cMET, PIM1, and NKIAMRE were identified as modulators of cell proliferation using co-immunoprecipitation assays known to those of skill in the art (*see, e.g.*, Harlow and Lane, *supra*). More specifically, PKC ζ , PLC β 1, cMET, PIM1, and NKIAMRE co-immunoprecipitated with cell cycle modulating proteins previously bound to a monoclonal antibody and thus were identified as modulators of cell proliferation. In particular, PKC ζ was identified using the monoclonal antibody ATM (specific for a nucleophosphoprotein involved in ataxia telangiectasia); PLC β 1 was identified using the monoclonal antibody p48 (specific for a subunit of the RB tumor suppressor gene); cMET was identified using the monoclonal antibody RbAp48 (specific for a fusion protein corresponding to amino acids 1-425 of human RbAp48); PIM1 was identified using the monoclonal antibody p21 (specific for the tumor suppressor gene p21); and NKIAMRE was identified using the monoclonal antibody RbAp48.

Example 2: Identification of Genes That Modulate Cell Proliferation Using Yeast Two Hybrid Assays

[0263] FAK, FAK2, CK2, FEN2, REV1, APE1, CDK3, CDC71, CDK7, CNK, PRL-3, STK2 (NEK4), and HBO1 were identified as modulators of cell proliferation using yeast two hybrid assays known to those of skill in the art (*see, e.g.*, Fields and Song, *Nature*, 340(6230):245 (1989). Briefly, two different haploid yeast strains of opposite mating types (*e.g.*, MAT α and MAT α) are generated. One strain contains a protein fused to the DNA binding domain (*i.e.*, binds to UASG) of the *Saccharomyces cerevisiae* transcriptional activator factor GAL4. The GAL4 DNA binding domain is typically placed upstream of reporter genes. Another strain contains a protein fused to the activation domain of GAL4. The strains are mated and transcription of the reporter gene is assayed. If the two proteins fused to the GAL4 domains interact to form a protein-protein complex, the DNA binding domain and the activation domain will reconstitute to form a functional transcriptional activator and reporter gene activity will be detected.

Example 3 Functional Characterization of Genes that Modulate the Cell Cycle Using Dominant Negative Mutants

[0264] Dominant negative mutants are used to study the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 on proliferation, the cell cycle, cell viability, and chemosensitization.

[0265] The anti-proliferative effects of dominant negative mutants are determined by GFP positivity assays. Briefly, Cell Tracker (CT) stained cells are infected with retroviruses engineered to express wild type and mutant PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1. The CT intensity of the GFP expressing population will be compared to the intensity of the GFP negative, uninfected population. Cells that stain brightly with the CT are identified as cell cycle arrested cells. Cells that stain dimly with CT are identified as proliferating cells.

[0266] Effects of dominant negative mutants on the cell cycle is measured by DAPI staining of transfected cells.

[0267] Effects of dominant negative mutants on cell viability is determined by monitoring the percent of GFP positive cells in an infected population at set intervals following infection.

[0268] Effects of dominant negative mutants on chemosensitization is determined by first treating transfected cells with chemotherapeutic agents such as, for example, bleomycin, etoposide, and cisplatin. After treatment with the chemotherapeutic agent, CT assays, DAPI staining assays, and GFP-positivity assays are conducted to assess the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 on proliferation, the cell cycle, cell viability, and chemosensitization.

[0269] Dominant negative mutants are used to determine the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 in different tumor types such as, for example, lung, colon, cervical, liver, kidney, uterine, or breast. Exemplary tumor cells lines include, A549 cells (lung, p53 wt) , H1299 (lung, p53 null), Hela (cervix, p53 deficient), Colo205 (colon, p53 mutant), and HCT116 (colon, p53 wt).

[0270] Dominant negative mutants are also used to determine the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 in tumor cells versus normal cells.

Exemplary tissue types include mammary epithelial cells, prostate epithelial cells, lung cells,
5 kidney cells, cervical cells and colon cells.

[0271] Dominant negative mutants were generated for CDC7L1, CNK, STK2, Hbo1, PIM1, APE1, CK2 or CK2 α , NKIAMRE, FEN1, and CDK3. The results are described in examples below.

Example 4 Functional Characterization of Genes that Modulate the Cell Cycle Using siRNA

10 [0272] Short interfering RNAs (siRNAs) are used to study the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 on proliferation and chemosensitization.

[0273] Four siRNAs are designed for each gene and transfected into A549 cells and Hela cells. mRNA reduction is tested using Taqman. siRNAs that induce greater than 70%
15 mRNA reduction are tested for anti-proliferative effects. Cy-3 labeled control siRNA, scrambled siRNAs, and the transfection reagent are used as controls.

[0274] siRNAs which show no independent anti-proliferative effects are analyzed for their ability to confer chemosensitization. 48 hours post transfection, cells are treated with chemotherapeutic agents, such as, for example, bleomycin, etoposide, and cisplatin. 48 hours
20 post-treatment, the IC50 of each chemotherapeutic agent is determined using BrdU ELISA and/or Cellomics image analysis which counts colonies and measures colony size.

[0275] siRNAs were designed for CDC7L1, CNK, Hbo1, PIM1, CK2 or CK2 α , and NKIAMRE. The results are discussed in examples below.

Example 5 Functional Characterization of Genes that Modulate the Cell Cycle Using Antisense Oligonucleotides

25 [0276] Antisense oligonucleotides are used to study the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 on proliferation and chemosensitization. Briefly, antisense oligonucleotides with a mixed phosphothiorate backbone are used to transfect A549
30 and Hela cells. Oligonucleotide concentrations of 50 nM or 100 nM are used to transfect the cells. Oligonucleotides which induce greater than 70% mRNA reduction in transfected cells

will be tested for anti-proliferative effects. Cell proliferation and viability assays are performed 48 hours post transfection with a BrdU ELISA and/or Cellomics image analysis which counts colonies and measures colony size. Antisense oligonucleotides which show no independent anti-proliferative effects are analyzed for their ability to confer

5 chemosensitization. 48 hours post transfection, cells are treated with chemotherapeutic agents, such as, for example, bleomycin, etoposide, and cisplatin. 48 hours post-treatment, the IC₅₀ of each chemotherapeutic agent is determined using BrdU ELISA and/or Cellomics image analysis.

[0277] Antisense oligonucleotides are used to determine the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 in different tumor types such as, for example, lung, colon, cervical, liver, kidney, uterine, or breast. Exemplary tumor cells lines include, A549 cells (lung, p53 wt), H1299 (lung, p53 null), Hela (cervix, p53 deficient), Colo205 (colon, p53 mutant), and HCT115 (colon, p53 wt).

15 [0278] Antisense oligonucleotides are also used to determine the effects of PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 in tumor cells versus normal cells. Exemplary tissue types include mammary epithelial cells, prostate epithelial cells, lung cells, kidney cells, cervical cells and colon cells.

20 Example 6 Identification of Genes that Modulate the Cell Cycle Using Proteomics

[0279] Proteomics assays are used to identify proteins that bind to PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1. Typically, the proteomics assays are performed after a functional screen to identify a gene of interest. Briefly, a potential binding partner is mixed
25 with a PKC- ζ , PLC- β 1, FAK, FAK2, CK2, cMET, FEN1, REV1, APE1, CDK3, PIM1, CDC7L1, CDK7, CNK, PRL-3, STK2 or NEK4, NKIAMRE, or HBO1 polypeptide bound to an affinity tag (*i.e.* a labeled monoclonal antibody). Complexes of the potential binding partner bound to the polypeptide are extracted, and analyzed, and the potential binding partner is identified.

Example 7: Assay for PLC β 1 Activity

[0280] PLC β 1 activity can be measured according to the method described in Nomoto *et al.*, *Jpn. J. Canc. Res.*, 89:1257-1266 (1998). Briefly, cell extracts are prepared and an appropriate amount of cell extract is suspended in reaction buffer (50 mM HEPES, pH 7.0, 100 mM NaCl, 1 mM CaCl₂, 0.15 mg/ml bovine serum albumin, and 1 mg/ml sodium deoxycholate) mixed with micelles of a substrate mixture of 1- α -phosphatidyl inositol and 1- α -phosphatidyl [2- ³H] inositol or a substrate mixture of 1- α -phosphatidyl inositol 4, 5-biphosphate and 1- α -phosphatidyl [2- ³H] inositol 4, 5-biphosphate at final concentrations of 100 μ M and 10⁴ dpm, respectively. After an appropriate incubation, the reaction is stopped, lipids are extracted from the reaction mixture and radioactivity in the aqueous fraction is detected with a liquid scintillation counter. Percent degradation of the labeled substrate is indicative of enzymatic activity.

Example 8: Assay for FAK2 Activity

[0281] FAK2 protein-tyrosine kinase activity can be measured according to the method described in Sasaki *et al.*, *J. Bio. Chem.*, 270(6):21206 (1995). Briefly, clarified cell lysates are incubated in 20 μ l of kinase assay buffer with 5 μ g/20 μ l of poly (Glu,Tyr), 5 μ Ci of [γ -³²P]ATP, 5 μ M unlabeled ATP, and 5 M MgCl₂. After an appropriate incubation, the reaction is stopped, and labeled substrate is separated by SDS-PAGE. ³²P-phosphorylated poly (Glu,Tyr) is visualized and quantitated by bioimaging analysis.

Example 9: Assay for CK2 Activity

[0282] CK2 activity can be measured according to the method described in Messenger *et al.*, *J. Biol. Chem.*, 277(25):23054 (2002). Briefly, cell extracts are incubated in 1 mM of a synthetic peptide substrate, RRRDDDSDDD in 20 mM Tris-HCl pH 7.5, 60 mM NaCl, 10 mM MgCl₂, 1 mM DTT, and 100 μ M γ -³²P-ATP. After an appropriate incubation, the reactions are stopped, run on SDS-PAGE, and phosphorylated proteins are detected by bioimaging analysis.

Example 10: Assay for cMET Activity

[0283] cMET activity can be measured according to the method described in Jeffers *et al.*, *Proc. Nat'l. Acad. Sci. USA* 94:11445 (1997). Briefly, cell lysates are prepared and immunoprecipitated using anti-Met SP260 (Santa Cruz Biotechnology) monoclonal antibody.

Immunoprecipitates are assessed for tyrosine kinase activity toward the exogenous substrate gastrin using a tyrosine kinase assay kit from Boehringer Mannheim.

Example 11: Assay for FEN1 Activity

[0284] FEN1 activity can be measured according to the method described in Tom *et al.*, *J. Biol. Chem.* 275(14):10498 (2000). Briefly, FEN1 is purified from cell extracts and incubated with appropriate amounts of oligonucleotide substrates and proliferating cell nuclear antigen in reaction buffer (30 mM HEPES pH 7.6, 5% glycerol, 40 mM KCL, 0.1 mg. ml bovine serum albumin, and 8 mM MgCl₂). After an appropriate incubation, the reactions are stopped, run on SDS-PAGE, and products are detected by bioimaging analysis.

Example 12: Assay for REV1 Activity

[0285] REV1 activity can be measured according to the method described in Zhang *et al.*, *Nuc. Acids Res.* 30(7):1630 (2002)). Briefly, REV1 is purified from cell extracts and incubated in reaction buffer (25 mM KH₂PO₄ pH 7.0, 5 mM MgCl₂, 10% glycerol, and 50 μ M of dNTPs (dATP, dCTP, dTTP, and dGTP) and 50 fmol of a DNA substrate containing a 5'-³²P labeled primer. After an appropriate incubation, the reactions are stopped, run on SDS-PAGE, and products are detected by bioimaging analysis.

Example 13: Assay for APE1 Activity

[0286] APE1 activity can be measured according to the method described in Tom *et al.*, *J. Biol. Chem.*, 276(52):48781 (2001). Briefly, APE1 is purified from cell extracts and incubated with appropriate amounts of oligonucleotide substrates in reaction buffer (30 mM HEPES pH 7.6, 5% glycerol, 40 mM KCL, 0.01% Nonidet P-40, 1 mg/ml bovine serum albumin, 8 mM MgCl₂, and 0.1 mM ATP). After an appropriate incubation, the reactions are stopped, run on SDS-PAGE, and products are detected by bioimaging analysis.

Example 14: Assay for CDC7L1 Activity

[0287] CDC7L1 activity can be measured according to the method described in Masai, *et al.*, *J. Biol. Chem.*, 275(37):29042 (2000). Briefly CDC7L1-ASK complexes are purified, mixed with [γ -³²P]ATP (1 μ Ci) and added to a reaction mixture containing MCM2-4-6-7- previously incubated with cdks and p27. After an appropriate incubation, the reactions are stopped, run on SDS-PAGE, and products are detected by bioimaging analysis.

Example 15: Assay for CNK Activity

[0288] CNK activity can be measured according to the method described in Ouyang *et al.*, *J. Biol. Chem.* 274:28646 (1997). Briefly, CNK is purified and assayed for kinase activity using one or more of the following substrates: casein (15 μ g/reaction), p53, GST-Cdc25A (5 μ g/reaction), GST-Cdc25B (5 μ g/reaction), His6-Cdc25c (5 μ g/reaction), GST-Cdc25C (1 μ g/reaction), or GST-Cdc25C^{S216A} (1 μ g/reaction).

Example 16 Assay for STK2 (NEK4) Activity

[0289] STK2 (NEK4) activity can be measured according to the method described in Hayashi *et al.*, *Biochem. Biophys. Res. Comm.*, 264:449 (1999). Briefly, STK2 complexes are immunoprecipitated, resuspended in kinase buffer (50 mM Tris-HCl pH 7.2, 3 mM MnCl₂) containing 10 μ Ci [γ -32P]ATP and 5 μ g of exogenous protein substrates. After an appropriate incubation, the reactions are stopped, the phosphorylated proteins are separated by SDS-PAGE, and detected by bioimaging analysis.

Example 17: Assay for HBO1 Activity

[0290] HBO1 can be measured according to the method described in Iizuka and Stillman, *J. Bio. Chem.*, 274(33):23027 (1999). Briefly, HBO1 polypeptides are immunoprecipitated from cell extracts and combined with a mixture recombinant *Xenopus* histone H3₂H4₂ tetramers (100 μ g/ml), human histone H2A:H2B (100 μ g/ml), and pmol of [³H]acetyl coenzyme A (11.2 Ci/mmol) in an appropriate volume of assay buffer (25 mM Tris-HCl, pH 8.5, 1 mM dithiothreitol, 0.5 mM EDTA, 5 mM sodium butyrate, 150 mM NaCl, 10% glycerol). After an appropriate incubation, the reactions are stopped, the phosphorylated proteins are separated by SDS-PAGE, and detected by Coomassie blue staining.

Example 18: Functional Characterization of CDC7LI Using Dominant Negative Mutants and siRNA Assays

[0291] CDC7LI was identified as a modulator of cellular proliferation in a yeast two hybrid assay using apoptin and GADD45. Vectors for the expression of CDC7LI fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and HeLa cells. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. As shown in Figure 20, expression of wild-type GFP-CDC7LI and mutant GFP-CDC7LI inhibited proliferation of A549 cells. The amino acid sequence of CDC7L mutants is shown in Figure 26.

[0292] CDC7LI mRNA expression was analyzed in tumor cell lines and in lung carcinomas and colon carcinomas. CDC7LI mRNA was overexpressed in tumor cell lines (*e.g.*, DU145, HCT116, SW620, Hela, and PC3) as compared to primary cell lines. See, *e.g.*, Figure 27. Figure 28 demonstrates that CDC7LI mRNA is expressed at higher levels in some lung
5 carcinomas compared to normal tissue from the same patient. Figure 29 demonstrates that CDC7LI mRNA is expressed at higher levels in some colon carcinomas compared to normal tissue from the same patient.

[0293] Two siRNAs induced greater than 50% reduction in mRNA expression when transfected into Hela cells. One of these siRNAs induced greater than 70% reduction in
10 mRNA expression. (Data not shown.)

Example 19 Functional Characterization of CNK Using Dominant Negative Mutants and siRNA Assays

[0294] CNK was identified as a modulator of cellular proliferation in a yeast two hybrid assay using DNAPK and F10. Vectors for the expression of CNK fused to the C-terminus of
15 GFP with a tetOff inducible gene expression system were used to transfect A549 cells and Hela cells. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. As shown in Figure 21, expression of wild-type CNK and mutant GFP-CNK inhibited proliferation of A549 cells. None of the siRNAs tested induced greater than 50% reduction in mRNA expression.

20 [0295] CNK mRNA expression was analyzed in tumor cell lines. CNK mRNA was overexpressed in tumor cell lines (*e.g.*, HCT116, PC3, A549, colo205, and H1299) as compared to primary cell lines. See, *e.g.*, Figure 30.

[0296] Wild type CNK and the CNK D146A mutant were fused to GST and produced in *E. coli*. (Data not shown.) Briefly, BL21(DE3) cells were transformed with either pDEST15-
25 CNK WT or CNK D146A and grown at 37°C to an OD600 of 0.6. Cultures were induced with 1 mM IPTG and then transferred to a 16°C shaking incubator for overnight incubation. After immobilization on glutathione-sepharose, proteins were eluted with 7.5 mM glutathione. The yield was approximately 0.5 mg/L for each protein.

[0297] The GST CNK fusions were tested for kinase activity in duplicate assays. See, *e.g.*,
30 Figure 31. The reaction buffer contained the following components: Reaction buffer: 10 mM Hepes, 10 μM ATP, 10 μM MnCl₂, 10 μCi γ-³²P ATP, 5 mM MgCl₂, 1 mM DTT, 1 mM

Na₃VO₄, 100 ng GST-CNK, 1.2 µg p53 or 10 µg MBP. Kinase reactions were incubated for thirty minutes at room temperature. The GST-CNK D146A mutant did not exhibit kinase activity. Wild type GST-CNK phosphorylated p53, maltose binding protein (MBP) and also exhibited autophosphorylation activity.

5 Example 20 Functional Characterization of STK2 Using Dominant Negative Mutants

[0298] STK2 was identified as a modulator of cellular proliferation in a yeast two hybrid assay using p73. STK2 is expressed as long and short isoforms (STK2L and STK2S). STK2L appears to be more highly expressed than STK2S in humans. See, *e.g.*, Figure 32.

10 [0299] STK2 mRNA expression was analyzed in tumor cell lines. STK2 mRNA was overexpressed in tumor cell lines (*e.g.*, HCT116 and PC3) as compared to primary cell lines. See, *e.g.*, Figure 33.

[0300] STK2 clones from a GFP C-terminal cDNA fusion library with a tetOff inducible gene expression system were used to transfect A549 cells and Hela cells. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. As shown in Figure 15 22, expression of wild-type STK2S inhibited proliferation of A549 cells and in Hela cells and expression of and mutant STK2S inhibited proliferation of A549 cells. Similar results are shown in Figure 34. Figure 35 shows that expression of GFP-STK2L inhibited proliferation of A549 and HeLa cells. Similar results were obtained for STK2L as shown in Figure 36. Using IRES vectors, expression of STK2L wild type and mutant proteins inhibited 20 proliferation in A549 cells. See, *e.g.*, Figure 37.

Example 21 Functional Characterization of Hbo1

[0301] Hbo1 mutants were constructed with the following mutations: Hbo1 G484E, Hbo1 L497S, and Hbo1 E508Q. Hbo1 mutants are shown in Figure 72. Both wild type and mutant Hbo1 proteins were localized to the cell nucleus. (Data not shown.)

25 [0302] The effect of Hbo1 expression on tumor cell lines was determined using cells that had been infected with a retrovirus that expressed Hbo1 wild type or mutant proteins. The Hbo1 E508Q mutant was antiproliferative in A549 cells (IRES only) and HeLa cells (GFP fusion and IRES construct) and had no effect in H1299 cells. Expression of the wild type Hbo1 protein and the other mutants had no effect on proliferation in this assay. See, *e.g.*, 30 Figures 38-40. Additional assays were performed using only sorted GFP positive cells as shown in Figure 41. Proliferation was measured using the CyQuant Cell Proliferation Assay

(Molecular Probes) which is based upon the fluorescence enhancement upon binding of a proprietary dye to cellular DNA. Using sorted cells, the Hbo1 E508Q mutant was strongly antiproliferative in A549 cells and HeLa cells. See, *e.g.*, Figures 42-43.

[0303] An Hbo1 siRNA caused greater than 50% reduction in mRNA expression when transfected into A549 cells or H1299 cells. The sequence of the Hbo1 siRNA is as follows: AACTGAGCAAGTGGTTGATTT. The Hbo1 siRNA had an antiproliferative effect when expressed in A549 or H1299 cells. See, *e.g.*, Figures 44-45.

Example 22 Functional Characterization of PIM1

[0304] PIM1 mRNA expression was analyzed in tumor cell lines and primary human tumors. PIM1 mRNA was overexpressed in tumor cell lines (*e.g.*, H1299, PC3, DU145, HCC1937, and MDA-MB-231) as compared to primary cell lines. See, *e.g.*, Figure 46. PIM1 appeared to be expressed at lower levels in breast carcinomas as compared to normal tissue from the same patient. See, *e.g.*, Figure 47. PIM1 also appeared to be expressed at lower levels in lung carcinomas as compared to normal tissue from the same patient. See, *e.g.*, Figure 48.

[0305] PIM1 mutants were constructed with the following mutations: Pim1 K67A and PIM1 D186N. PIM1 mutants are shown in figure 73.

[0306] Vectors for the expression of PIM1 fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and H1299 cells. Similar experiments were done using an IRES vector. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. Figure 49 shows that in A549 cells, expression of wild type PIM1, but not the mutants, was antiproliferative. Figure 50 shows that in H1299 cells GFP fused wild type PIM1 was antiproliferative. Using IRES constructs, expression of wild type PIM1 and the PIM1 mutants was antiproliferative in H1299 cells.

[0307] A PIM1-specific siRNA caused greater than 50% reduction in mRNA expression when transfected into A549 cells, HeLa cells, or H1299 cells. The sequence of the PIM1 siRNA is as follows: AAAACTCCGAGTGAAGTGGTC. The PIM1 siRNA had an antiproliferative effect when expressed in A549, HeLa cells, or H1299 cells. See, *e.g.*, Figures 51-53. In primary HUVEC cells the PIM1-specific siRNA caused greater than 50% reduction in mRNA expression and had an antiproliferative effect. See, *e.g.*, Figure 54.

[0308] Wild type and mutant PIM1 proteins were expressed in Phoenix cells and assayed for kinase activity using Histone H1 as a substrate. Wild type and mutant PIM1 proteins were fused to GFP and also had a myc tag. Wild type and mutant PIM1 proteins were immunoprecipitated using an anti-myc antibody and the immune complexes were assayed for kinase activity using 20 μ l of kinase buffer + 0.5 μ L of γ -³²P ATP (3000 Ci/mmol). Kinase buffer contained 20 mM Tris, pH 7.5; 50 mM NaCl; 10 mM MgCl₂; 2 mM MnCl₂; 1 mM NaF; and 1 mM Na₃VO₄. Kinase reactions were incubated at room temperature for one hour and analyzed by SDS-PAGE and autoradiography. Wild type PIM1 exhibited kinase activity, while the mutant PIM1 proteins did not. (Data not shown.) Western blot analysis was used to show the equivalent amounts of wild type and mutant PIM1 proteins were assayed. (Data not shown.)

Example 23 Functional Characterization of APE1

[0309] APE1 mutants were constructed with the following mutations: APE1 E96A, APE1 D210A, and APE1 C65A.

[0310] Subcellular localization studies demonstrated that APE1 mutant and wild type proteins were localized to the cell nucleus in A549 cells. (Data not shown.)

[0311] Vectors for the expression of APE1 fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and H1299 cells. APE1 mutants were also expressed. Similar experiments were done using an IRES vector. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. In A549 cells, expression of wild type and mutant APE1 proteins had no apparent effect on proliferation. See, *e.g.*, Figure 55. Similar results were obtained in H1299 cells. See, *e.g.*, Figure 56. However, in primary HMEC cells, expression of both wild type APE1 and the APE1 D210A mutant was antiproliferative. See, *e.g.*, Figure 57.

[0312] Expression of the APE1 D210A mutant in A549 cells sensitized the cells to methyl methanesulfonate (MMS) treatment. At 72 hours after infection, A549 cells were treated with 3mM MMS for 60 min. Survival curves are shown in Figure 58.

[0313] Expression of APE1 wildtype and the APE1 C65A mutant were protective in A549, HeLa, and H1299 cells treated with bleomycin. See, *e.g.*, Figures 59-60. These results are consistent with those published by Robertson *et al.*, *Cancer Res.* 61:2220-5 (2001), showing that overexpression of Ape1 in the tumor line NT2 confers resistance to bleomycin treatment.

Example 24 Functional Characterization of Casein kinase II alpha (CK2 α or CK2)

[0314] CK2 α mRNA expression was analyzed in tumor cell lines and primary human cell lines and results are shown in Figure 61. CK2 α dominant negative mutants are shown in Figure 62. Subcellular localization studies demonstrated that CK2 α mutant and wild type proteins were localized to the cell nucleus and concentrated in punctuate areas outside the nucleus in A549 cells. (Data not shown.) Neither CK2 α wild type or mutant protein expression was antiproliferative in A549 or H1299 cells. (Data not shown.)

[0315] A CK2 α -specific siRNA caused greater than 50% reduction in mRNA expression when transfected into H1299 cells. The sequence of the CK2 α -specific siRNA (also know as CK2) is as follows: AACATTGAATTAGATCCACGT. The CK2 α siRNA had an antiproliferative effect when expressed in H1299 cells. See, *e.g.*, Figure 63. The same CK2 α siRNA reduced mRNA in HeLa cells but did not appear to effect cell proliferation. (Data not shown.)

Example 25 Functional Characterization of NKIAMRE

[0316] NKIAMRE mRNA expression was analyzed in tumor cell lines. NKIAMRE mRNA was overexpressed in tumor cell lines (*e.g.*, H1299, PC3, DU145, HCT116, and MDA-MB-231) as compared to primary cell lines. See, *e.g.*, Figure 64. Dominant negative mutants of NKIAMRE were generated and are shown in Figure 65. Subcellular localization studies demonstrated that NKIAMRE mutant and wild type proteins were localized to the cell cytoplasm in A549 cells. (Data not shown.)

[0317] Vectors for the expression of NKIAMRE fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and H1299 cells. NKIAMRE mutants were also expressed. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. In A549 cells and H1299 cells, expression of wild type and mutant NKIAMRE proteins had no apparent effect on proliferation. See, *e.g.*, Figure 74.

[0318] NKIAMRE-specific siRNA caused greater than 50% reduction in mRNA expression when transfected into H1299 cells or HeLa cells, but did not appear to affect proliferation in either cell line. Data not shown.

Example 26 Functional Characterization of FEN1

[0319] Dominant negative mutants of FEN1 were generated and are shown in Figure 66. Vectors for the expression of FEN1 fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and H1299 cells. GFP fusions

were also made using the FEN1 dominant negative mutants. Similar experiments were done using an IRES vector. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. Figure 67 shows that in A549 cells, expression of mutant FEN1, but not the wild type, was antiproliferative. Figure 68 shows that in H1299 cells, expression of the FEN1 dominant negative mutants was also antiproliferative.

Example 27 Functional Characterization of CDK3

[0320] Dominant negative mutants of CDK3 were generated and are shown in Figure 69.

Vectors for the expression of CDK3 fused to the C-terminus of GFP with a tetOff inducible gene expression system were used to transfect A549 cells and H1299 cells. GFP fusions

were also made using the CDK3 dominant negative mutants. Similar experiments were done using an IRES vector. Cell proliferation was measured using Cell Tracker assays, *i.e.*, detecting GFP positivity. Figure 70 shows that in A549 cells, expression of either wild type CDK3 or mutant CDK3 proteins had no apparent antiproliferative effect. Figure 71 shows that in H1299 cells, expression of either wild type CDK3 or mutant CDK3 proteins had no apparent antiproliferative effect.

[0321] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

- 1 1. A method for identifying a compound that modulates cell cycle
2 arrest, the method comprising the steps of:
3 (i) contacting a cell comprising a target polypeptide or fragment thereof or
4 inactive variant thereof, selected from the group consisting of flap structure specific
5 endonuclease 1 (FEN1), protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1),
6 protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2
7 (CK2), cMET tyrosine kinase (cMET), REV1 dCMP transferase (REV1),
8 apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1
9 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7
10 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine
11 phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent
12 serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), or fragment thereof
13 with the compound, the target polypeptide encoded by the complement of a nucleic acid
14 that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having
15 an amino acid sequence selected from the group consisting of SEQ ID NO:14, 2, 4, 6, 8,
16 10, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36; and
17 (ii) determining the chemical or phenotypic effect of the compound upon
18 the cell comprising the target polypeptide or fragment thereof or inactive variant thereof,
19 thereby identifying a compound that modulates cell cycle arrest.
- 1 2. The method of claim 1, wherein the chemical or phenotypic effect
2 is determined by measuring enzymatic activity selected from the group consisting of
3 nuclease activity, kinase activity, lipase activity, transferase activity, phosphatase activity,
4 and acetylase activity.
- 1 3. The method of claim 1, wherein the chemical or phenotypic effect
2 is determined by measuring cellular proliferation.
- 1 4. The method of claim 3, wherein the cellular proliferation is
2 measured by assaying fluorescent marker level or DNA synthesis.
- 1 5. The method of claim 4, wherein DNA synthesis is measured by ^3H
2 thymidine incorporation, BrdU incorporation, or Hoescht staining.

- 1 6. The method of claim 4, wherein the fluorescent marker is selected
2 from the group consisting of a cell tracker dye or green fluorescent protein.
- 1 7. The method of claim 1, wherein modulation is activation of cell
2 cycle arrest.
- 1 8. The method of claim 1, wherein modulation is activation of cancer
2 cell cycle arrest.
- 1 9. The method of claim 1, wherein the host cell is a cancer cell.
- 1 10. The method of claim 9, wherein the cancer cell is a breast, prostate,
2 colon, or lung cancer cell.
- 1 11. The method of claim 9, wherein the cancer cell is a transformed
2 cell line.
- 1 12. The method of claim 11, wherein the transformed cell line is A549,
2 PC3, H1299, MDA-MB-231, MCF7, or HeLa.
- 1 13. The method of claim 9, wherein the cancer cell is p53 null or
2 mutant.
- 1 14. The method of claim 9, wherein the cancer cell is p53 wild-type.
- 1 15. The method of claim 1, wherein the polypeptide is recombinant.
- 1 16. The method of claim 1, wherein the polypeptide is encoded by a
2 nucleic acid comprising a sequence of SEQ ID NO:13, 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23,
3 25, 27, 29, 31, 33, or 35.
- 1 17. The method of claim 1, wherein the compound is an antibody.
- 1 18. The method of claim 1, wherein the compound is a small organic
2 molecule.
- 1 19. The method of claim 1, wherein the compound is an antisense
2 molecule.

1 20 . The method of claim 1, wherein the compound is a peptide.

1 21. The method of claim 20, wherein the peptide is circular.

1 22 . The method of claim 1, wherein the compound is an siRNA
2 molecule.

1 23. A method for identifying a compound that modulates cell cycle
2 arrest, the method comprising the steps of:

3 (i) contacting a cell comprising a target polypeptide or fragment thereof or
4 inactive variant thereof, selected from the group consisting of flap structure specific
5 endonuclease 1 (FEN1), protein kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1),
6 protein tyrosine kinase 2 (FAK), protein tyrosine kinase 2b (FAK2), casein kinase 2
7 (CK2), cMET tyrosine kinase (cMET), REV1 dCMP transferase (REV1),
8 apurinic/aprimidinic nuclease 1 (APE1), cyclin dependent kinase 3 (CDK3), PIM1
9 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent kinase 7
10 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein tyrosine
11 phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent
12 serine threonine kinase (NKLAMRE), or histone acetylase (HBO1), or fragment thereof
13 with the compound, the target polypeptide encoded by the complement of a nucleic acid
14 that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having
15 an amino acid sequence selected from the group consisting of SEQ ID NO:14, 2, 4, 6, 8,
16 10, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36; and

17 (ii) determining the physical effect of the compound upon the target
18 polypeptide or fragment thereof or inactive variant thereof; and

19 (iii) determining the chemical or phenotypic effect of the compound upon
20 a cell comprising the target polypeptide or or fragment thereof or inactive variant thereof,
21 thereby identifying a compound that modulates cell cycle arrest.

1 24. A method of modulating cell cycle arrest in a subject, the method
2 comprising the step of administering to the subject a therapeutically effective amount of a
3 compound identified using the method of claim 1.

1 25. The method of claim 24, wherein the subject is a human.

1 26. The method of claim 25, wherein the subject has cancer.

1 27. The method of claim 24, wherein the compound is a small organic
2 molecule.

1 28. The method of claim 24, wherein the compound is an antisense
2 molecule.

1 29. The method of claim 24, wherein the compound is an antibody.

1 30. The method of claim 24, wherein the compound is a peptide.

1 31. The method of claim 30, wherein the peptide is circular.

1 32. The method of claim 24, wherein the compound is an siRNA
2 molecule.

1 33. The method of claim 24, wherein the compound inhibits cancer cell
2 proliferation.

1 34. A method of modulating cell cycle arrests in a subject, the method
2 comprising the step of administering to the subject a therapeutically effective amount of a
3 target polypeptide or fragment thereof or inactive variant thereof, selected from the group
4 consisting of flap structure specific endonuclease 1 (FEN1), protein kinase C ζ (PKC- ζ),
5 phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein tyrosine kinase
6 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), REV1 dCMP
7 transferase (REV1), apurinic/apyrimidinic nuclease 1 (APE1), cyclin dependent kinase 3
8 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin dependent
9 kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein
10 tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin
11 dependent serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), or
12 fragment thereof with the compound, the target polypeptide encoded by the complement
13 of a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a
14 polypeptide having an amino acid sequence selected from the group consisting of SEQ ID
15 NO:14, 2, 4, 6, 8, 10, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36.

1 35. A method of modulating cell cycle arrest in a subject, the method
2 comprising the step of administering to the subject a therapeutically effective amount of a
3 nucleic acid encoding a target polypeptide or fragment thereof or inactive variant thereof,
4 selected from the group consisting of flap structure specific endonuclease 1 (FEN1), protein
5 kinase C ζ (PKC- ζ), phospholipase C- β 1 (PLC- β 1), protein tyrosine kinase 2 (FAK), protein
6 tyrosine kinase 2b (FAK2), casein kinase 2 (CK2), cMET tyrosine kinase (cMET), REV1
7 dCMP transferase (REV1), apurinic/aprimidinic nuclease 1 (APE1), cyclin dependent
8 kinase 3 (CDK3), PIM1 kinase (PIM1), cell division cycle 7 kinase (CDC7L1), cyclin
9 dependent kinase 7 (CDK7), cytokine inducible kinase (CNK), potentially prenylated protein
10 tyrosine phosphatase (PRL-3), serine threonine kinase 2 (STK2) or (NEK4), cyclin dependent
11 serine threonine kinase (NKIAMRE), or histone acetylase (HBO1), or fragment thereof with
12 the compound, the target polypeptide encoded by the complement of a nucleic acid that
13 hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an
14 amino acid sequence selected from the group consisting of SEQ ID NO:14, 2, 4, 6, 8, 10, 12,
15 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36.

1 36. A CK2-specific siRNA molecule comprising the sequence
2 AACATTGAATTAGATCCACGT, wherein the siRNA molecule is from 21 to 30 nucleotide
3 base pairs in length.

1 37. The CK2-specific siRNA molecule of claim 36 consisting of the
2 sequence AACATTGAATTAGATCCACGT and its complement as active portion.

1 38. A method of inhibiting expression of a CK2 gene in a cell, the method
2 comprising contacting the cell with a CK2-specific siRNA molecule comprising the sequence
3 AACATTGAATTAGATCCACGT, wherein the siRNA molecule is from 21 to 30 nucleotide
4 base pairs in length.

1 39. A PIM1-specific siRNA molecule comprising the sequence
2 AAAACTCCGAGTGAAGTGGTC, wherein the siRNA molecule is from 21 to 30
3 nucleotide base pairs in length.

1 40. The PIM1-specific siRNA molecule of claim 39 consisting of the
2 sequence AAAACTCCGAGTGAAGTGGTC and its complement as active portion.

1 41. A method of inhibiting expression of a PIM1 gene in a cell, the method
2 comprising contacting the cell with a PIM1-specific siRNA molecule comprising the
3 sequence AAAACTCCGAGTGAAGTGGTC, wherein the siRNA molecule is from 21 to 30
4 nucleotide base pairs in length.

1 42. An Hbo1-specific siRNA molecule comprising the sequence
2 AACTGAGCAAGTGGTTGATTT, wherein the siRNA molecule is from 21 to 30 nucleotide
3 base pairs in length.

1 43. The Hbo1-specific siRNA molecule of claim 42 consisting of the
2 sequence AACTGAGCAAGTGGTTGATTT and its complement as active portion.

1 44. A method of inhibiting expression of an Hbo1 gene in a cell, the
2 method comprising contacting the cell with an Hbo1-specific siRNA molecule comprising
3 the sequence AACTGAGCAAGTGGTTGATTT, wherein the siRNA molecule is from 21 to
4 30 nucleotide base pairs in length.

SEQ ID NO:1
Size: 2164
DNA PKC-_γ

1/84

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121  tgtgaggaag  tgagagacat  gtgtcgtctg  caccagcagc  acccgctcac  cctcaagtgg
181  gtggacagcg  aaggtgaccc  ttgcacgggtg  tcctcccaga  tggagctgga  agaggctttc
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781  gggagctacg  ccaaggttct  cctggtgcgg  ttgaagaaga  atgaccaaata  ttacgccatg
841  aaagtgtgta  agaaagagct  ggtgcatgat  gacgaggata  ttgactgggt  acagacagag
901  aagcagctgt  ttgagcaggc  atccagcaac  cccttctctg  tcggattaca  ctctgcttcc
961  cagacgacaa  gtcggttggt  cctggtcatt  gagtacgtca  acggcgggga  cctgatgttc
1021  cacatgcaga  ggcagaggaa  gctccctgag  gagcacgcca  ggttctacgc  ggccgagatc
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1621  gacgaactag  gtctggacaa  ctttgacaca  cagttcacca  gcgagcccg  gcagctgacc
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2041  gctgtcatgc  ggtttccaag  gtgcacattt  tccacggaaa  cagaactcga  tgactgacc
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2161  aaaa

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SEQ ID NO:2
Size: 592
PRT PKC-_γ

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121  RWRKLYRANG  HLFQAKRFNR  RAYCGQCSER  IWGLARQGYR  CINCKLLVHK  RCHGLVPLTC
181  RKHMDSVMPS  QEPPVDDKNE  DADLPSEETD  GIAYISSSRK  HDSIKDDSED  LKPVIDGMDG
241  IKISQGLGLQ  DFDLIRVIGR  GTYAKVLLVR  LKKNDQIYAM  KVVKKELVHD  DEDIDWVQTE
301  KHVFEQASSN  PFLVGLHSCF  QTTSRLFLVI  EYVNGGDLMF  HMQRQRKLPE  EHARFYAAEI
361  CIALNFLHER  GIIYRDLKLD  NVLLDADGHI  KLTGYGMCKE  GLGPGDTTST  FCGTPNYIAP
421  EILRGEEYGF  SVDWWALGVL  MFEMMAGRSP  FDIITDNPDM  NTEDYLFQVI  LEKPIRIPRF
481  LSVKASHVLK  GFLNKDPKER  LGCRPQTGFS  DIKSHAFFRS  IDWDLLEKKQ  ALPPFPQPII
541  DDYGLDNFOT  QFTSEPVQLT  PDDEDAIKRI  DQSEFEGFEY  INPLLLSTEE  SV

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FIG. 1

2/84

SEQ ID NO: 3
Size: 3663
DNA PLC-1

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121 ccaattatth tgaggactga cctcaggga tttttctttt actggacaga tcaaaacaag
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481 aagctgcaag tcactccaga agggcgatatt cctctcaaaa acatatatcg cttgttttca
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601 gattcaatac ctcaagaaga tttcactcca gaagtgtaca gagttttcct caacaacctt
661 tgccctcgac ctgaaattga taacatcttt tcagaatttg gtgcaaaaag caaacatat
721 cttaccgttg atcagatgat ggattttatc aaccttaagc agcgagatcc tggccttaat
781 gaaatacttt atccacctct aaaacaagag caagtccaag tattgattga gaagtatgaa
841 cccaacaaca gcctcgccag aaaaggacaa atatcagtgg atgggttcat gcgctatctg
901 agtggagaag aaaacggagt cgtttcacct gaaaaactgg atttgaatga agacatgtct
961 cagccctttt ctactatttt cattaattcc tcgcacaaca cctacctcac agctggccaa
1021 ctggctggaa actcctctgt tgagatgtat cgccaagtgc tctgtctggg ttgtcgctgt
1081 gtggagctgg actgctggaa gggacggact gcagaagagg aacctgtcat caccatggc
1141 ttcaccatga caactgaaat atctttcaag gaagtgatag aagcaattgc ggagtgtgca
1201 tttaaagactt cactttttcc aattctcctt tcgtttgaga accatgtgga tccccaaag
1261 cagcaagcca agatggcgga gtactggcga ctgatctttg gggatggcct cctctggag
1321 cccctggaaa aatatccact ggaatctgga gttcctcttc caagccctat ggatttaatg
1381 tataaaatth tggtgaaaaa taagaagaaa tcacacaagt catcagaagg aagcggcaaa
1441 aagaagctct cagaacaagc ctccaacacc tacagtgact cctccagcat gttcgagccc
1501 tcatccccag gagccggaga agctgatacg gaaagtgcag acgacgatga tgatgatgac
1561 tgtaaaaaat cttcaatgga tgaggggact gctggaagtg aggtatggc cacagaagaa
1621 atgtctaata tgggtgaacta tattcagcca gtcaagtttg agtcatttga aatttcaaaa
1681 aaaaagaaata aaagttttga aatgtcttcc ttcgtggaaa ccaaaggact tgaacaactc
1741 accaagtctc cagtgggaatt tgtagaatat aacaaaatgc agcttagcag gatatatcca
1801 aaaggaacac gtgtggattc atccaactat atgcctcagc tcttctggaa tgcaggttgt
1861 cagatggtgg cacttaattt ccagacaatg gacctggcta tgcaaataaa tatggggatg
1921 tatgaataca acgggaagag tggctacaga ttgaagccag agttcatgag gaggcctgac
1981 aagcattttg atccatttac tgaaggcatc gtagatggga tagtggcaaa cactttgtct
2041 gttaagatta tttcaggcca gtttcttctt gataagaaaag ttgggactta cgtggaagta
2101 gatatgtttg gtttgctgtg ggatacaagg aggaaggcat ttaagaccaa aacatcccaa
2161 ggaaatgctg tgaatcctgt ctgggaagaa gaacctattg tgttcaaaaa ggtggttctt
2221 cctactctgg cctgttttgag aatagcagtt tatgaagaag gaggtaaatt cattggccac
2281 cgtatcttgc cagtgcgaagc cattcgccca ggctatcact atatctgtct aaggaatgaa
2341 aggaaccagc ctctgaagct gcctgctgtc tttgtctaca tagaagtgaag agactatgtg
2401 ccagacacat atgcagatgt catcgaagct ttatcaaaccc caatccgata tgtgaacctg
2461 atggaacaga gagctaagca attggctgct ttgacactgg aagatgaaga agaagtaaag
2521 aaagaggctg atcctggaga aacaccatca gaggtcccaa gtgaagcgag aacgactcca
2581 gcagaaaatg ggtgaaatca cactacaacc ctgacacca agccaccctc ccaggctctc
2641 cacagccagc cagctccagg ttctgtaaaag gcacctgcca aaacagaaga tcttattcag
2701 agtgtcttaa cagaagtggg agcacagacc atcgaagaac taaagcaaca gaaatcgtht
2761 gtgaaacttc aaaagaaaca ctacaaaagaa atgaaaagacc tgggttaagag acaccacaag
2821 aaaaccactg accttatcaa agaacacact accaagtata atgaaattca cactgactac
2881 ttgagaagga gagccgcttt ggaaaagtcc gccaaaaagg acagtaagaa aaaatcggaa
2941 cccagcagcc ctgatcatgg ttcataacag attgagcaag acctcgctgc tctggatgct
3001 gaaatgaccc aaaagttaat agacttgaag gacaaaacaac agcagcagct gcttaattct
3061 cggcaagaac agtattatag tgaaaaatag cagaagcgag aacatattaa actgcttatt
3121 caaaagttga cggatgtcgc agaagagtggt cagaacaatc agttaagaa gctcaagaa

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FIG. 2

SUBSTITUTE SHEET (RULE 26)

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3181 atctgtgaga aagaaaagaa agaattaaag aagaaaatgg ataaaaagag gcaggagaag
3241 ataacagaag ctaaatccaa agacaaaagt cagatggaag aggagaagac agagatgac
3301 cggcatata tccaggaagt ggtgcagtat atcaagaggc tagaagaagc gcaaagtaaa
3361 cggcaagaaa aactcgtaga gaaacacaag gaaatacgtc agcagatcct ggatgaaaag
3421 cccaagctgc aggtggagct ggagcaagaa taccaagaca aattcaaaag actgcccctc
3481 gagattttgg aattcgtgca ggaagccatg aaaggaaaaga tcagtgaaga cagcaatcac
3541 ggttctgccc ctctctccct gtcctcagac cctggaaaag tgaaccacaa gactccctcc
3601 agtgaggagc tgggaggaga catcccagga aaagaatttg atactcctct gtgaatgctc
3661 ctg

```

SEQ ID NO:4

Size: 1216

PRT PLC-1

```

1  MAGAQPGVHA LQLKPVCSVD SLKKGTKFVK WDDSTIVTP IILRTDPQGF FFYWTDQNK
61  TELLDSLVLK DARCGRHAKA PKDPKLRELL DVGNIQRLEQ RMITVVYGPD LVNISHNLV
121 AFQEEVAKEW TNEVFSLATN LLAQNMSRDA FLEKAYTKLK LQVTPEGRIP LKNYIRLFSA
181 DRKRIVETALE ACSLPSSRND SIPQEDFTPE VYRVFLNNLC PRPEIDNIFS EFGAKSKPYL
241 TVDQMMDFIN LKQRDPRLNE ILYPPLKQEQ VQVLEKYEP NNSLARKGQI SVDGFMRYLS
301 GEENGVSPE KLDLNEDEMSQ PLSHYFINSS HNTYLTAGQL AGNSSVEMYR QVLLSGCRV
361 ELDCWKGRTA EEPVITHGF TMTTEISFKE VIEAIAECF KTSPFPILLS FENHVDSPKQ
421 QAKMAEYCRL IFGDALLMEP LEKYPLESGV PLPSPMDLMY KILVKNKKKS HKSSEGSCK
481 KLSEQASNTY SDSSSMFEP SPGAGEADTE SDDDDDDDDC KKSMDDEGTA GSEAMATEEM
541 SNLVNYIQPV KFESFEISK RNSKFEMSSF VETKGLEQLT KSPVEFVEYN KMQLSRIYPK
601 GTRVDSSNYM POLFWNAGCQ MVALNFQTM LAMQINMGMY EYNGKSGYRL KPEFMRRPDK
661 HFDPFTEGIV DGIVANTLSV KIISGFSLSD KKVGTVEVD MFGLPVDTRR KAFKTKTSQG
721 NAVNPVWEEE PIVFKKVLP TLACLRIVY EEGGKFIGHR ILPVQAIRPG YHYICLRNER
781 NQPLTLPAVF VYIEVKDYP DTYADVIEAL SNPIRYVNL EQRAQLAAL TLEDEEVVK
841 EADPGETPSE APSEARTTPA ENGVNHTTTL TPKPPSQALH SQPAPGSVKA PAKTEDLIQS
901 VLTEVEAQT EELKQKSFV KLQKKHYKEM KDLVKRHHK TTDLIKEHTT KYNEIQNDYL
961 RRAALEKSA KKDSKKKSEP SSPDHGSSTI EQDLAALDAE MTQKLIDLK KQQQQLNLR
1021 QEQYYSEKYQ KREHIKLLIQ KLTDVAECEQ NNQLKKLKEI CEKEKKELKK KMDKKRQEKI
1081 TEAKSKDKSQ MEEKTE MIR SYIQEVVQYI KRLEEAQSKR QEKLVEKHKE IRQQILDEKP
1141 KLQVELEQY QDKFKRLPLE ILEFVQEAMK GKISEDNSHG SAPLSLSSDP GKVNHKTPSS
1201 EELGGDIPGK EFDTP

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FIG. 2
(CONT.)

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SEQ ID NO:5
Size: 3052
DNA FAK

```

1  ccggtgtgaa  ggccatgagt  gattactggg  ttgttggaaa  gaagtctaac  tatgaagtat
61  tagaaaaaga  tgttggttta  aagcgatttt  ttcctaagag  tttactggat  tctgtcaagg
121  ccaaaacact  aagaaaactg  atccaacaaa  catttagaca  atttgccaac  cttaatagag
181  aagaaagtat  tctgaaattc  tttagatcc  tgtctccagt  ctacagattt  gataaggaat
241  gcttcaagtg  tgctcttggt  tcaagctgga  ttatttcagt  ggaactggca  atcggcccag
301  aagaaggaat  cagttaccta  acggacaagg  gctgcaatcc  cacacatctt  gctgacttca
361  ctcaagtgca  aaccattcag  tattcaaaca  gtgaagacaa  ggacagaaaa  ggaatgctac
421  aactaaaaat  agcaggtgca  cccgagcctc  tgacagtga  ggcaccatcc  ctaaccattg
481  cggagaatat  ggctgacct  atagatgggt  actgccggct  ggtgaatgga  acctcgagct
541  cttttatcat  cagacctcag  aaagaagggt  aacgggcttt  gccatcaata  ccaaagttgg
601  ccaacagcga  aaagcaaggc  atgcggacac  acgccgtctc  tgtgtcagaa  acagatgatt
661  atgctgagat  tatagatgaa  gaagatactt  acaccatgcc  ctcaaccagg  gattatgaga
721  ttcaagaga  aagaatagaa  cttggacgat  gtattggaga  aggccaatct  ggagatgtac
781  atcaaggcat  ttatatgagt  ccagagaatc  cagctttggc  ggttgcaatt  aaaacatgta
841  aaaactgtac  ttccgacagc  gtgagagaga  aatttcttca  agaagcctgc  cattacacat
901  ctttgactg  gaattgggtg  agatatataa  gtgaccta  tgttgatgcc  tgcccagacc
961  ccaggaatgc  agagttaaca  atgcgtcagt  ttgaccatcc  tcatattgtg  aagctgattg
1021  gagtcacac  agagaatcct  gtctggataa  tcatggagct  gtgcacactt  ggagagctga
1081  ggtcattttt  gcaagtaagg  aaatacagtt  tggatctagc  atctttgatc  ctgtatgcct
1141  atcagcttag  tacagctctt  gcatatctag  agagcaaaag  atttgtacac  agggacattg
1201  ctgctcggaa  tgttctgggt  tcctcaaatg  atttgttaaa  attaggagac  tttggattat
1261  ccgatatat  ggaagatagt  acttactaca  aagcttccaa  aggaaaattg  cctattaaat
1321  ggatggctcc  agagtcaatc  aattttcgac  gttttacctc  agctagtga  gtatggatgt
1381  ttggtgtgtg  tatgtgggag  atactgatgc  atggtgtgaa  gccttttcaa  ggagtgaaga
1441  acaatgatgt  aatcggtcga  attgaaaatt  gggaaagatt  accaatgcct  ccaaattgtc
1501  ctccctacct  ctacagcctt  atgacgaat  gctgggccta  tgacccagc  aggcggccca
1561  ggtttactga  acttaaagct  cagctcagca  caatcctgga  ggaagagaag  gctcagcaag
1621  aagagcgcac  gaggatggag  tccagaagac  aggccacagt  gtccctgggac  tccggagggt
1681  ctgatgaagc  accgcccag  cccagcagac  cgggttatcc  cagtcagagg  tccagcgaag
1741  gattttatcc  cagcccacag  cacatggtac  aaaccaatca  ttaccagggt  tctggctacc
1801  ctggttcaca  tggaatcaca  gccatggctg  gcagcatcta  tccaggtcag  gcattctctt
1861  tggaccaaac  agattcatgg  aatcatagat  ctcaaggagat  agcaattgtg  cagcccaatg
1921  tggaggactc  tacagtattg  gacctgcgag  ggattgggca  agtgttgcca  acccatctga
1981  tggaagagcg  tctaattcga  cagcaacagg  aaatggaaga  agatcagcgc  tggctggaaa
2041  aagaggaaa  atttctgatt  ggaaaccaac  atatatatca  gcctgtgggt  aaaccagatc
2101  ctgcagctcc  accaaagaaa  ccgcctcgcc  ctggagctcc  cggctcatctg  ggaagccttg
2161  ccagcctcag  cagccctgct  gacagctaca  acgagggtgt  caagcttcag  cccaggaaa
2221  tcagccccc  tccactgccc  aacctggacc  ggtcgaaatga  taagggtgac  gagaatgtga
2281  cgggcctggt  gaaagctgtc  atcgagatgt  ccagtaaaat  ccagccagcc  ccaccagagg
2341  agtatgtccc  tatggtgaag  gaagtcggct  tggccctgag  gacattattg  gccactgtgg
2401  atgagaccat  tcccctccta  ccagccagca  cccaccgaga  gattgagatg  gcacagaagc
2461  tattgaactc  tgacctgggt  gagctcatca  acaagatgaa  actggcccag  cagtatgtca
2521  tgaccagcct  ccagcaagag  taaaaaaagc  aaatgctgac  tgccgctcac  gccctggctg
2581  tggatgccaa  aaacttactc  gatgtcattg  accaagcaag  actgaaaatg  cttgggcaga
2641  cgagaccaca  ctgagcctcc  cctaggagca  cgtcttgcta  ccctcttttg  aagatgttct
2701  ctagccttcc  accagcagcg  aggaattaac  cctgtgtcct  cagtcgccag  cactcacagc
2761  tccaactttt  ttgaatgacc  atctgggtga  aaaatctttc  tcatataagt  ttaaccacac
2821  tttgatttgg  gttcattttt  tgttttgttt  ttttcaatca  tgatattcag  aaaaatccag
2881  gatccaaaat  gtggcgtttt  tctaagaatg  aaaattatat  gtaagctttt  aagcatcatg
2941  aagaacaatt  tatgttcaca  ttaagatacg  ttctaagggt  ggatggccaa  ggggtgacat
3001  ctttaattcct  aaactacctt  agctgcatag  tggaaaggga  gagccggaat  tc

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FIG. 3

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SEQ ID NO:6
Size: 879
PRT FAK

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1  MSDYWVVGKK SNYEVLEKDV GLKRFFPKSL LDSVKAKTLR KLIQQTFRQF ANLNREESIL
61  KFFEILSPVY RFDKECFKCA LGSSWIISVE LAIGPEEGIS YLTDKGCNPT HLAFTQVQT
121 IQYNSSEDKD RKGMLQLKIA GAPEPLTVTA PSLTIAENMA DLIDGYCRLV NGTSQSFIIR
181 PQKEGERALP SIPKLANSEK QGMRTHAVSV SETDDYAEII DEEDTYTMPs TRDYEIQRER
241 IELGRCIGEG QFGDVHQGIY MSPENPALAV AIKTCKNCTS DSVREKFLQE ACHYTSLHWN
301 WCRYISDPNV DACPDPRNAE LTMROFDHPH IVKLIGVITE NPVWIIMELC TLGELRSFLQ
361 VRKYSLDLAS LILYAYQLST ALAYLESKRF VHRDIAARNV LVSSNDCVKL GDFGLSRYME
421 DSTYYKASKG KLPIKWMAPE SINFRRTSA SDVWMFVCM WEILMHGVKP FQGVKNNDVI
481 GRIENGERLP MPPNCPPTLY SLMTKCWAYD PSRRPRFTEL KAQLSTILEE EKAQQEERM
541 MESRRQATVS WDSGGSDEAP PKPSRPGYPS PRSSEGFYPS PQHMQTNHY QVSGYPGSHG
601 ITAMAGSIYP GQASLLDQTD SWNHRSQEIA MWQPNVEDST VLDLRGIGQV LPTHLMEEERL
661 IRQQQEMEED QRWLEKEERF LIGNQHIYQP VGKPDPAAPP KKPPRPGAPG HLGSLASLSS
721 PADSYNegVK LQPQEISPPP TANLDRSNDK VYENVTLGLVK AVIEMSSKIQ PAPPEEYVPM
781 VKEVGLALRT LLATVDETIP LLPASTHREI EMAQKLLNSD LGELINKMKL AQQYVMTSLQ
841 QEYKKQMLTA AHALAVIDAKN LLDVIDQARL KMLGQTRPH

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FIG. 3
(CONT.)

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SEQ ID NO:7

Size: 4089

DNA FAK2

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1  gaattcogtc agccctttta ctcagccaca gcctccggag ccgttgacaca cctacctgcc
61  cggccgactt acctgtactt gccgccgtcc cgggtcacct ggccggtgccc gaggagtagt
121  cgctggagtc cggccctccc tgggactgca atgtgccgat cttagctgct gcctgagagg
181  atgtctgggg tgtccgagcc cctgagtcga gtaaagtggg gcacgttacg ccggcctgaa
241  ggccctgcag agcccatggt ggtggtacca gtagatgtgg aaaaggagga cgtgcgtatc
301  ctcaaggtct gcttctatag caacagcttc aatcctggga aaaacttcaa actggtcaaa
361  tgcactgtcc agacggagat ccgggagatc atcacctcca tctgtctgag cgggcggatc
421  gggcccaaca tccggttgcc tgagtgtctat gggctgaggc tgaagcacat gaagtccgat
481  gagatccact ggctgcaccc acagatgacg gtgggtgagg tgcaggacaa gtatgagtgt
541  ctgcaogtgg aagccgagtg gaggtatgac cttcaaatacc gctacttgcc agaagacttc
601  atggagagcc tgaaggagga caggaccacg ctgctctatt ttaccaaca cgtccggaac
661  gactacatgc agcgctacgc cagcaagggtc agcgagggca tggccctgca gctgggctgc
721  ctggagctca ggcggttctt caaggatatg cccacacatg cacttgacaa gaagtccaac
781  ttogagctcc tagaaaagga agtggggctg gacttgtttt tcccaaagca gatgcaggag
841  aacttaaagc ccaaacagtt ccggaagatg atccagcaga ccttcacgca gtacgcctcg
901  ctcagggagg aggagtgcgt catgaagttc ttcaacactc tcgcccgtt cgccaacatc
961  gaccaggaga cctaccgctg tgaactcatt caaggatgga acattactgt ggacctgggtc
1021  attggcccta aagggatccg ccagctgact agtcaggacg caaagccac ctgacctggcc
1081  gagttcaagc agatcaggtc catcagggtc ctcccgctgg aggagggcca ggcagtactt
1141  cagctgggca ttgaagggtc ccccagggcc ttgtccatca aaacctcatc cctagcagag
1201  gctgagaaca tggctgacct catagacggc tactgccggc tgcagggtga gcaccaaggc
1261  tctctcatca tccatcctag gaaagatggt gagaagcggg acagcttgcc ccagatcccc
1321  atcctaaacc tggaggcccg gcgtccccc cctcagaga gctgcagcat agagtcagac
1381  atctacgcag agattcccg aaaaaccctg cgaaggcccg gaggtccaca gtatggcatt
1441  gcccggtgaag atgtggtcct gaatcgtatt cttggggaag gcttttttgg ggaggtctat
1501  gaaggtgtct acacaaatca taaaggggag aaaatcaatg tagctgtcaa gacctgcaag
1561  aaagactgca ctctggacaa caaggagaag ttcatgagcg aggcagtgat catgaagaac
1621  ctcgaccacc cgcacatcgt gaagctgatc ggcattcatt aagaggagcc cacctggatc
1681  atcatggaat tgtatcccta tggggagctg ggccactacc tggagcggaa caagaactcc
1741  ctgaagggtg tcaccctcgt gctgtactca ctgcagatat gcaaagccat ggcctacctg
1801  gagagcatca actgctgca cagggacatt gctgtccgga acatcctggt ggcctccctt
1861  gagtgtgtga agctggggga ctttgggtct tcccgttaca ttgaggacga ggactattac
1921  aaagcctctg tgactcgtct ccccatcaaa tggatgtccc cagagtccat taacttccga
1981  cgcttcacga cagccagtga cgtctggatg ttcccgctgt gcatgtggga gatcctgagc
2041  tttgggaagc agcccttctt ctggctggag aacaaggatg tcatcgggt gctggagaaa
2101  gggacccggc tgcccaagcc tgatctctgt ccacoggtcc tttataacct catgaaccgc
2161  tgctgggact acgaccccg tgaccggccc cgttccaccg agctggtgtg cagcctcagt
2221  gacgtttatc agatggagaa ggacattgcc atggagcaag agaggaatgc tgcctaccga
2281  acccccaaaa tcttggagcc cacagccttc caggaacccc cacccaagcc cagccgacct
2341  aagtacagac cccctccgca aaccaacctc ctggtcccaa agctgcagtt ccaggttcct
2401  gaggggtctg gtgccagtc tctacgctc accagcccta tggagtatcc atctccggtt
2461  aactcactgc acaccccacc tctccacggt cacaatgtct tcaaagccca cagcatgggg
2521  gaggaggact tcatccaacc cagcagccga gaagaggccc agcagctgtg ggaggctgaa
2581  aaggtcaaaa tgcggcaaat cctggacaaa cagcagaagc agatggtgga ggactaccag
2641  tggctcaggc aggaggagaa gtccctggac cccatgggtt atatgaatga taagtcccca
2701  ttgacgccag agaaggaggt cggtacacct gagttcacag ggccccca gaagcccccg
2761  aggtggggc cacagtccat ccagcccaca gctaacctgg accggaccga tgacctggtg
2821  tacctcaatg tcatggagct ggtgcgggcc gtgctggagc tcaagaatga gctctgcag
2881  ctgcccccg agggctacgt ggtggtggtg aagaatgtgg ggctgacct cgggaagctc
2941  atcgggagcg tggatgatct cctgccttcc ttgcogtcat cttcacggac agagatcgag
3001  ggcaccaga aactgctcaa caaagacctg gcagagctca tcaacaagat gcggtggcg
3061  cagcagaacg ccgtgacctc cctgagtgag gagtgcaga ggcagatgct gacggcttca
3121  cacaccctgg ctgtggacgc caagaacctg ctgcagctg tggaccaggc caaggttctg
3181  gccaatctgg cccaccacc tgcagagtga cggagggtg gggccacctg cctgcgtctt
3241  ccgcccctgc ctgccatgta cctcccctgc cttgctgttg gtcatgtggg tcttccaggg

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FIG. 4

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3301 agaaggccaa ggggagtcac cttcccttgc cactttgcac gacgccctct cccaccccct
3361 acccctggct gtactgtctca ggctgcagct ggacagaggg gactctgggc tatggacaca
3421 ggggtgacggt gacaaagatg gctcagaggg ggactgtctgc tgccctggcca ctgctcccta
3481 agccagcctg gtccatgcag ggggctcctg ggggtgggga ggtgtcacat ggtgccccta
3541 gctttatata tggacatggc aggccgattt gggaaccaag ctattccttt cccttcctct
3601 tctcccctca gatgtccctt gatgcacaga gaagctgggg aggagctttg ttttcggggg
3661 tcaggcagcc agtgagatga gggatgggcc tggcattctt gtacagtgtg tattgaaatt
3721 tatttaaatgt gaggtttggt ctggactgac agcatgtgcc ctctgaggg aggaccagg
3781 cacagtccag gaacaagcta attgggagtc caggcacagg atgctgtgtt gtcaacaaac
3841 caagcatcag ggggaagaag cagagagatg cggccaagat aggaccttgg gccaaatccg
3901 ctctcttctt gccctctttt ctctttcttc ctttaactttt ccttgctttt ccctcttttc
3961 ttactcctcc tctttctctc cccacacccc attctcatct gcacccttct tttctcatgt
4021 gtttgcataa acattctttt aacttctttc tatttgactt gtggttgaat taaaattgtc
4081 ccatttgca

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SEQ ID NO:8
Size: 1009
PRT FAK2

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1 MSGVSEPLSR VKLGTLLRPE GPAEPMVVVP VDVEKEDVRI LKVCFYNSNF NPGKNFKLVK
61 CTVQTEIREI ITSILLSGRI GPNIRLAECY GLRLKHKMSD EIHWHLPQMT VGEVQDKYEC
121 LHVEAEWRYD LQIRYLPEDF MESLKEDRTT LLYFYQQLRN DYMQRYSASKV SEGMAQLQGC
181 LELRRFFKDM PHNALDKSN FELLEKEVGL DLFFPKQMQE NLKPKQFRKM IQQTFQQYAS
241 LREEECVMKF FNTLAGFANI DQETYRCELI QGWNITVDLV IGPKGIRQLT SQDAKPTCLA
301 EFKQIRSIRC LPLEEGQAVL QLGIEGAPQA LSIKTSSLAE AENMADLIDG YCRLQGEHOG
361 SLIIHPRKDG EKRNSLPQIP MLNLEARRSH LSESCSIESD IYAEIPDETLL RRPGGPQYGI
421 AREDVVLNRI LGEGFFGEVY EGVYTNHKGE KINVAVKTC KDCTLDNKEK FMSEAVIMKN
481 LDHPHIVKLI GIIEEPTWI IMELYPYGEL GHYLERNKNS LKVLTLVLYS LQICKAMAYL
541 ESINCVHRDI AVRNLVASE ECVKLGDFGL SRYIEDEDY KASVTRLPIK WMSPEINFR
601 RFTTASDVWM FAVCMWEILS FGKQFFFWLE NKDVIGVLEK GDRLPKPDL C PPVLYTLMTR
661 CWDYDPSDRP RFTELVCSL S DVIQMEKDIA MEQERNARYR TPKILEPTAF QEPKPSRP
721 KYRPPPTNL LAPKLQFQVP EGLCASSPTL TSPMEYPSPV NSLHTPPLHR HNVFKRHSR
781 EEDFIQPSR EEAQQLWEAE KVKMRQILDK QKQMVEDYQ WLRQEEKSLD PMVYMNDKSP
841 LTPEKEVGYL EFTGPPQKPP RLGAQSIQPT ANLDRTDDL V YLNMELVRA VLELKNELCQ
901 LPPEGVYVVV KNVGLTLRKL IGSVDDLLPS LPSSSRTEIE GTQKLLNKDL AELINKMRLA
961 QQNAVTSLS ECKRQMLTAS HTLAVDAKNL LDAVDQAKVL ANLAHPAE

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FIG. 4
(CONT.)

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SEQ ID NO:9
Size: 2195
DNA CK2

```

1 aggggagagc ggccgcccgc gctgcccgtt ccaccacagt ttgaagaaaa caggtctgaa
61 acaaggtctt acccccagct gcttctgaac acagtgactg ccagatctcc aaacatcaag
121 tccagctttg tccgccaacc tgtctgacat gtcgggaccc gtgccaagca gggccagagt
181 ttacacagat gttaatcac acagacctcg agaatactgg gattacgagt cacatgtggt
241 ggaatgggga aatcaagatg actaccagct ggttcgaaaa ttaggccgag gtaaatacag
301 tgaagtattt gaagccatca acatcacaaa taatgaaaaa gttgttggtt aaattctcaa
361 gccagtaaaa aagaagaaaa ttaagcgtga aataaagatt ttggagaatt tgagaggagg
421 tcccaacatc atcacactgg cagacattgt aaaagaccct gtgtcacgaa ccccgccctt
481 ggtttttgaa cacgtaaaaa acacagactt caagcaattg taccagacgt taacagacta
541 tgatattoga ttttacctgt atgagattct gaaggccctg gattattgtc acagcatggg
601 aattatgcac agagatgtca agccccataa tgtcatgatt gatcatgagc acagaaagct
661 acgactaata gactgggggtt tggctgagtt ttatcatcct ggccaagaat ataattgtccg
721 agttgcttcc cgatacttca aaggctcctga gctacttgta gactatcaga tgtacgatta
781 tagtttggtat atgtggagtt tgggttgat gctggcaagt atgatcttcc ggaaggagcc
841 atttttccat ggacatgaca attatgatca gttggtgagg atagccaagg ttctggggac
901 agaagattta tatgactata ttgacaaaaa caacattgaa ttagatccac gtttcaatga
961 tatcttgggc agacactctc gaaagcgatg ggaacgcttt gtccacagtg aaattcagca
1021 ccttgtcagc cctgaggcct tggatttctt ggacaaaactg ctgcatatg accaccagtc
1081 acggcttact gcaagagagg caatggagca cccctatttc tacactgttg tgaaggacca
1141 ggctcgaatg ggttcaccta gcatgccagg gggcagtagc cccgtcagca gcgccaatat
1201 gatgtcaggg atttcttcag tgccaacccc ttcaccctt ggacctctgg caggctcacc
1261 agtgattgct gctgccaaac cccttgggat gctgttcca gctgccgctg gcgctcagca
1321 gtaacggccc tatctgtctc ctgatgcctg agcagaggtg ggggagtcca cctctcctt
1381 gatgcagctt gcgcctggcg gggaggggtg aaacacttca gaagcacctg gtctgaaccg
1441 ttgcttgttg atttatagta gttcagtcat aaaaaaaaaa ttataatagg ctgattttct
1501 tttttctttt tttttttaac tcgaactttt cataactcag gggattccct gaaaaattac
1561 ctgcaggttg aatatttcat ggacaaaattt tttttctcc cctcccaaatt ttagttcctc
1621 atcacaaaag aacaaagata aaccagcctc aatcccggt gctgcattta ggtggagact
1681 tcttccattt cccaccattg ttctccacc gtccacact ttagggggtt ggtatctcgt
1741 gctcttctcc agagattaca aaaatgtagc ttctcagggg aggcaggaa gaaaggaaagga
1801 aggaaagaag gaaggagga cccaatctat aggagcagtg gactgcttgc tggctcgctta
1861 catcacttta ctccataagc gcttcagtg ggttatccta gtggctcttg tggaagtgtg
1921 tcttagttac atcaagatgt tgaaaaatcta cccaaaatgc agacagatac taaaaacttc
1981 tgttcagtaa gaatcatgtc ttactgatct aaccctaaat ccaactcatt tatactttta
2041 tttttagttc agtttaaaat gttgatacct tccctcccag gctccttacc ttggtctttt
2101 ccctgttcat ctcccaacat gctgtgtctc atagctggtt ggagagggaa ggcaaaatct
2161 ttcttagttt tctttgtctt ggccattttg aattc

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SEQ ID NO:10
Size: 391
PRT CK2

```

1 MSGPVPSRAR VYTDVNTHRP REYWDYESHV VEWGNQDDYQ LVRKLGRGKY SEVFEAINIT
61 NNEKVVVKIL KPVKKKKIKR EIKILENLRG GPNIITLADI VKDPVSRTPA LVFEHVNNND
121 FKQLYQTLTD YDIRFYMYEI LKALDYCHSM GIMHRDVKPH NVMIDHEHRK LRLIDWGLAE
181 FYHPGQYENV RVASRYFKGP ELLVDYQMYD YSLDMWSLGC MLASMIFRKE PFFHGHNDYD
241 QLVRIAKVLG TEDLYDYIDK YNIELDPREN DILGRHSRKR WERFVHSENQ HLVSPALDF
301 LDKLLRYDHQ SRLTAREAME HPYFYTVVKD QARMGSSSMP GGSTPVSSAN MSGGISSVPT
361 PSPLGPLAGS PVIAAANPLG MPVPAAGAQA Q

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FIG. 5

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SEQ ID NO:11

Size: 4626

DNA cMET

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1  gaattccgcc  ctgcgcgcgc  gcggcgcccc  gagcgctttg  tgagcagatg  cggagccgag
61  tggagggcgc  gagccagatg  cggggcgaca  gctgaacttg  tgagaggagg  cggggaggcg
121  cggagcgcgc  gtgtggctct  tgcgcgcgtg  acttctccac  tggttcctgg  gcaccgaaag
181  ataaacctct  cataatgaag  gcccccgtg  tgettgcacc  tggcatcctc  gtgctcctgt
241  ttaccttggg  gcagaggagc  aatggggagt  gtaaagaggc  actagcaaag  tccgagatga
301  atgtgaatat  gaagtatcag  cttcccaact  tcaccgcgga  aacacccatc  cagaatgtca
361  ttctacatga  gcatcacatt  ttcttgggtg  ccactaacta  catttatgtt  ttaaattgag
421  aagaccttca  gaaggttgct  gagtacaaga  ctgggcctgt  gctggaacac  ccagattgtt
481  tcccatgtca  ggactgcagc  agcaaagcca  atttatcagg  aggtgttttg  aaagataaca
541  tcaacatggc  tctagttgtc  gacacctact  atgatgatca  actcattagc  tgtggcagcg
601  tcaacagagg  gacctgccag  cgacatgtct  tccccacaa  tcatactgct  gacatacagt
661  cggagggttca  ctgcatattc  tccccacaga  tagaagagcc  cagccagtgt  cctgactgtg
721  tgggtgagcgc  cctgggagcc  aaagtccttt  catctgtaaa  ggaccgggtc  atcaacttct
781  ttgtaggcaa  taccataaat  tcttcttatt  tcccagatca  tccattgcat  tcgatatcag
841  tgagaaggct  aaaggaaaac  aaagatgggt  ttatgttttt  gacggaccag  tcctacattg
901  atgtttttacc  tgagttcaga  gattctttacc  ccattaagta  tgtccatgcc  tttgaaagca
961  acaattttat  ttacttcttg  acgggtccaaa  gggaaaactc  agatgctcag  acttttcaca
1021  caagaataat  caggttctgt  tccataaact  ctggattgca  ttctacatg  gaaatgcctc
1081  tggagtgtat  tctcacagaa  aagagaaaaa  agagatccac  aaagaaggaa  gtgtttaata
1141  tacttcaggc  tgcgtatgtc  agcaagcctg  gggcccagct  tgctagacaa  ataggagcca
1201  gcctgaatga  tgacattctt  ttccgggtgt  tgcacaaaag  caagccagat  tctgccgaac
1261  caatggatcg  atctgccatg  tgtgacttcc  ctatcaaata  tgtcaacgac  tcttcaaca
1321  agatgcgtcaa  caaaaacaa  gtgagatgtc  tccagcattt  ttacagacc  aatcctgagc
1381  actgctttta  taggacactt  ctgagaaatt  catcaggctg  tgaagcgcgc  cgtgatgaat
1441  atcgaacaga  gtttaccaca  gctttgcagc  gcgttgactt  attcatgggt  caattcagcg
1501  aagtcctctt  aacatctata  tccaccttca  ttaaaggaga  cctcaccata  gctaattctg
1561  ggacatcaga  gggtcgcttc  atgcaggttg  tggtttctcg  atcaggacca  tcaacccctc
1621  atgtgaattt  tctcctggac  tcccattcag  tgtctccaga  agtgattgtg  gagcatatag
1681  taaacaaaaa  tggctacaca  ctggttatca  ctgggaagaa  gatcacgaag  atcccattga
1741  atggcttggg  ctgcagacat  ttccagtcct  gcagtcaatg  cctctctgcc  ccaacctttg
1801  ttcagtgtgg  ctggtgccac  gacaaatgtg  tgcgatcgga  ggaatgcctg  agcgggacat
1861  ggactcaaca  gatctgtctg  cctgcaatct  acaagggttt  cccaaatagt  gcaacccctg
1921  aaggaggggac  aaggctgacc  atatgtggct  gggacttttg  atttcggagg  aataataaat
1981  ttgatttaaa  gaaaactaga  gttctccttg  gaaatgagag  ctgcaccttg  actttaagtg
2041  agagcacgat  gaatacattg  aaatgcacag  ttggctcctg  catgaataag  catttcaata
2101  tgtccataat  tatttcaaat  ggccacggga  caacacaata  cagtacattc  tcctatgtgg
2161  atcctgtaat  aacaagtatt  tcgccgaaat  acggctctat  ggctggtggc  actttactta
2221  ctttaactgg  aaattaccta  aacagtggga  attctagaca  catttcaatt  ggtggaaaaa
2281  catgtacttt  aaaaagtgtg  tcaaacagta  ttcttgaatg  ttatacccca  gcccaaacca
2341  tttcaactga  gtttgctgtt  aaattgaaaa  ttgacttagc  caaccgagag  acaagcatct
2401  tcagttaccg  tgaagatccc  attgtctatg  aaattcatcc  aaccaaatct  tttattagta
2461  cttggtggaa  agaacctctc  aacattgtca  gttttctatt  ttgctttgcc  agtgggtggg
2521  gcacaataac  aggtgttggg  aaaaacctga  attcagttag  tgtcccgaga  atggtcataa
2581  atgtgcatga  agcaggaagg  aactttacag  tggcatgtca  acatcgctct  aattcagaga
2641  taatctgttg  taccactcct  tccctgcaac  agctgaatct  gcaactcccc  ctgaaaacca
2701  aagccttttt  catgttagat  gggatccttt  ccaaataact  tgatctcatt  tatgtacata
2761  atcctgtgtt  taagcctttt  gaaaagcccg  tgatgatctc  aatgggcaat  gaaaatgtac
2821  tggaaattaa  gggaaatgat  attgacctg  aagcagttaa  aggtgaagtg  ttaaagttg
2881  gaaataagag  ctgtgagaat  atacacttac  attctgaagc  cgttttatgc  acgggtcccca
2941  atgacctgct  gaaattgaac  agcgagctaa  atatagagtg  gaagcaagca  atttcttcaa
3001  cgtccttgg  aaaagtaata  gttcaaccag  atcagaatct  cacaggattg  attgctgggtg
3061  ttgtctcaat  atcaacagca  ctgttattac  tacttgggtt  tttcctgtgg  ctgaaaaaga
3121  gaaagcaaat  taaagatctg  ggcagtgaat  tagttcgcta  cgatgcaaga  gtacacactc

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FIG. 6

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3181 ctcatttggga taggcttgta agtgcccgaag gtgtaagccc aactacagaa atggtttcaa
3241 atgaatctgt agactaccga gctacttttc cagaagatca gtttccta atcatctcaga
3301 acgggttcatg ccgacaagtgc cagtatcctc tgacagacat gtcccccata ctaactagtgc
3361 gggactctga tataatccagc ccattactgc aaaatactgt ccacattgac ctcagtgtctc
3421 taaatccaga gctggtccag gcagtgcagc atgtagtgat tgggcccagt agcctgattg
3481 tgcatttcaa tgaagtcata ggaagagggc attttggttg tgtatatcat gggactttgt
3541 tggacaatga tggcaagaaa attcactgtg ctgtgaaatc cttgaacaga atcactgaca
3601 taggagaagt ttcccaattt ctgaccgagg gaatcatcat gaaagatttt agtcatccca
3661 atgtcctctc gctcctggga atctgcctgc gaagtgaagg gtctccgctg gtggtcctac
3721 catacatgaa acatggagat cttcgaaatt tcattcgaaa tgagactcat aatccaactg
3781 taaaagatct tattggcttt ggtcttcaag tagccaaagc gatgaaatat cttgcaagca
3841 aaaagtttgt ccacagagac ttggctgcaa gaaactgtat gctggatgaa aaattcacag
3901 tcaagggttgc tgattttggt cttgccagag acatgtatga taaagaatac tatagtgtac
3961 acaacaaaac aggtgcaaag ctgccagtga agtggatggc tttgaaaagt ctgcaaaactc
4021 aaaagtttac caccaagtca gatgtgtggt cctttggcgt cgtcctctgg gagctgatga
4081 caagaggagc cccaccttat cctgacgtaa acacctttga tataactgtg tacttggttc
4141 aaggggagaag actcctacaa ccogaatact gccagacccc cttatatgaa gtaatgctaa
4201 aatgctggca ccctaaagcc gaaatgcgcc catccttttc tgaactgggtg tcccgatat
4261 cagcgatctt ctctactttc attggggagc actatgtcca tgtgaacgct acttatgtga
4321 acgtaaaatg tgtcgctccg tatcctttctc tgttgtcatc agaagataac gctgatgatg
4381 aggtggacac acgaccagcc tcttctctggg agacatcata gtgctagtag tgctcgaag
4441 caacagttcca cactttgtcc aatggttttt tcaactgcctg accttataaa ggccatcgat
4501 attctttgct ccttgccata ggacttgtat tgttatttaa attactggat tctaaggaat
4561 ttcttatctg acagagcatc agaaccagag gcttgggtccc acaggccagg gaccaatgag
4621 ctgcag

```

SEQ ID NO:12

Size: 1408

PRT cMET

```

1 MKAPAVLAPG ILVLLFTLVQ RSNGECKEAL AKSEMNVNMK YQLPNFTAET PIQNVILHEH
61 HIFLGATNYI YVLNEEDLQK VAHEYKTGPVL EHPDCFPQCD CSSKANLSGG VWKDNINMAL
121 VVDITYDDQL ISCGSVNRGT CQRHVFPNNH TADIQSEVHC IFSPQIEEPS QCPDCVVSAL
181 GAKVLSSVKD RFINFFVGNT INSSYFPDHP LHSISVRLK ETKDGFMLT DQSYIDVLPE
241 FRDSYPIKYV HAFESNNFIY FLTQVRETLD AQTFFHTRIIR FCSINSLGHS YMEMPLECIL
301 TEKRKKRSTK KEVFNILQAA YVSKPGAQLA RQIGASLND ILFGVFAQSK PDSAEPMDRS
361 AMCAFPKIYV NDFFNKIVNK NNVRCLOHFI GPNHEHCFNR TLLRNSSGCE ARRDEYRTEF
421 TTALQRVDLF MGQFSEVLLT SISTFIKGLD TIANLGTSEG RFMQVVVSRS GPSTPHVNFL
481 LDSHPVSPEV IVEHTLNQNG YTLVITGKKI TKIPLNGLGC RHFQSCSQCL SAPPFVQCGW
541 CHDKCVRSEE CLSGTWTQOI CLPAIYKVFP NSAPLEGGR LTICGWDFGF RRNNKFDLKK
601 TRVLLGNESC TLTLSESTMN TLKCTVGPAM NKHFNMSSII SNGHGTQYS TFSYVDPVIT
661 SISPKYGPMA GGTLTLTLGN YLNSGNSRHI SIGGKTCTLK SVSNSILECY TPAQTISTEF
721 AVKLKIDLAN RETSIFSIRE DPIVYEHPT KSFISTWWKE PLNIVSFLFC FASGGSTITG
781 VGKNLNSVSV PRMVINVHEA GRNFTVACQH RSNSEIICCT TPLSLQQLNLQ LPLKTKAFFM
841 LDGILSKYFD LIYVHNPFVK PFEKPMISM GNENVLEIKG NDIDPEAVKG EVLVGNKSC
901 ENIHLHSEAV LCTVPNDLLK LNSELNIEWK QAISSVTLGK VIVQPDQNTF GLIAGVVSIS
961 TALLLLLGFF LWLKKRKQIK DLGSELVRYD ARVHTPHLDR LVSARSVSPT TEMVSNESVD
1021 YRATFPEDQF PNSSQNGSCR QVQYPLTDM PILTSGDSI SSPLLQNTVH IDLSALNPCL
1081 VQAVQHVIG PSSLIHFNE VIGRGHFGCV YHGTLLDNDG KKIHCARKSL NRITDIGEVS
1141 QFLTEGIIMK DFSHPNVLSL LGICLRSEGS PLVVLPMKH GDLRNFIRNE THNPTVKDLI
1201 GFGLQVAKAM KYLASKKFVH RDLAARNCML DEKFTVKVAD FGLARDMYDK EYYSVHNKTI
1261 AKLPQKWMAL ESLQTQKFTT KSDVWSEFVV LWELMTRGAP PYPDVNTFDI TVYLLQGRRL
1321 LQPEYCPDPL YEVMKLCWHP KAEMRPSFSE LVSRIASIFS TFIGEHYVHV NATYVNVKCV
1381 APYPSLLSSE DNADDEVDR PASFWETS

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FIG. 6
(CONT.)

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SEQ ID NO:13
Size: 3350
DNA FEN1

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1  cacagtccac tctgtcaggg ttttaaggcag gaaaaacatg ctcatTTTTga tggtaatatatt
61  ctTcctttctc aacattccat ttctcctggc aaatttcatg gatcccagat gcttttggaa
121 aataaatttg aatgaaatca aggatgaagt ccttgggatg acttgTtcooT tcatccttga
181 aacagttcag aagactatgg acaaagatta tttcaaccag actctgaatg tcctaaatac
241 aactacaaac cacaatatg ccttggcatt ggcttttaca gtggatgaaa tcaacaggaa
301 tcctgatctt ttaccaaata tgtctctgat tataaaatac aatttgggTc attgtgatgg
361 aaaaactgta acaactctat ccgatttatt taatccaaat aatcatctcc atttccccaa
421 ttattttatgt aatgaaggga ttatgtgttt ggTtctgctt acaggaccac attggagagc
481 atctttatat ctctggatat ccgtgtatgt ctacctgtct ccacatttcc ttcagctttc
541 ctatggacct ttctactcca tcttcagtga taatgaacaa tatccttata tctatcagat
601 gggcccaaaag gactcatcac tagcattggc aatggTctcc ttcataaattt acttcaagtg
661 gaactgggtt gggctattta tctcagatga tgatcaaggc aatcaatttc tctcagagtt
721 gaaaaaagag agccaaacca aggatatttg ctttgccttt gtgaacatga tatcagtcag
781 tgatgtttca tactatcata aaactgaaat gtactacaac caaattgtga tgtcatccac
841 aaaggTtatt atcatttatg gggaacaaa cagtattatt gaattgagct tcagaatgtg
901 gTcatctcca gttaaacaga gaatatgggt caccacaaaa caatttgatt gccctaccag
961 taagagagac ttaactcatg gcacattcta tgggaccctt acatttctac accactatgg
1021 tgagattttct ggcttttaaaa attttgtaca gacacggTac aatctcagaa gcacagattt
1081 atatctagta atgccagagt ggaaatatTT taactatgaa gcctcagcat ctaactgtaa
1141 aatactgaga aactatttat ccaatatctc actggaatgg ctaatggaac agaaaatttga
1201 catgtcattt agtgattata gTcaacaocat atacaatgct gtatatgcca ttgtctatgc
1261 actccatgag aagaatctgc aagaagtTga aaatcaggca ataaacaatg cgaagagaga
1321 aaatactcac tgcttgaagc taaactcatt tctgagaaag acccacttca ctaattctct
1381 tgggaacaga gtaattatga aacagagaga agtagtgcat ggagactata atattgttca
1441 catgtggaat ttctcacaaac gccttgggat taaggTgaag ataggacaat tcagcccaca
1501 ttttccacag gTcaacagT ccatacctcag tgtgcagtgc agattgccat cctggattca gaagaatctg
1561 tagaaagatg atggcagcct gctgttttTg ttgcaacccc tgccctgaaa atgaaatttc
1621 gaaggaggaa atgggtgTat tttgggtctt cgtgaagcac catgacactc ctattgtgaa
1681 taatgagacg atgggtgTat tttgggtctt cgtgaagcac ctcatgttct gttttctgtg
1741 ggccaataac agaatcctca gctaactatt aatcgtgtca ctcatgttct gttttctgtg
1801 ctcccttttc ttcatTggct atcctaacag agcaacctgt atcttacagc aaatcacatt
1861 tggaaTcttc ttactgtTg ctatttccac agttctggcc aaaacaatca ctgtggTtct
1921 ggcttttcaa gtcacagacc caggaagaca attaagaatc tttttggTat cggggacacc
1981 caactacatt attcccatat gttccctatt gcaatgtatt ctgtgtgcaa tctggctagc
2041 agtttctcct ccctttgtTg atattgatga acactctgag catggccaca tcatcattgt
2101 gtgcaacaag ggctccatta ctgcattcta ctgtgtcctg ggatactTgg cctgcctggc
2161 ctttTgaagc ttactatag ctttcttggc aaagaacctg cctgacacat tcaacgaagc
2221 caagtTcttg accttcagca tgctagtgtt ctgcgctgtc tgggtcacct tccctccctg
2281 ctaccatagc accaaggTca aggtcatggt tgcTgtggag atcttctcca tcttggcact
2341 tagtgcaggg atgctgggat gcatcTttgc acccaaagTt tacatcattt taatgagacc
2401 agacagaaat tcgatccaca aaatcaggga gaaatcatat ttctgaaaag gtatttcagg
2461 aattctgtca aatgtaaagt tgatacatac accccaaata tttagTtaca gagcatatat
2521 ctagtTtttag aatcactctc actggTtccT ctagtTaaGc atagaagtac catatgtact
2581 gatctTgcat atgtTgtcta taaaatctta caatcattca tttgcttagt atcttctgga
2641 agaagTaaaa ttttcaaata actagtacaa ttttattcat tattttgcTt tcatgaggat
2701 ttcccccTgg taacttcaaa taaattttat aagtcagtTg aatatataac cttacataga
2761 aagtgaGttc taggacagac agggattata catagaaaca aactaactaa aaatcaacaa
2821 agatgaaatc agaacacatt ttcttatttc cagttaggaac acatactTga cagaatactg
2881 tctttttttc agctgctctt taagatatTg gccaatagtc taagctgaaa atgttcttta
2941 tctactctca aatacaaaaa tattatatcc aacaatggac agaatctgag aactcctgtg
3001 gttgagTtag ggaatagTtg gaagatactg agaaggaggt gaccatagg aatacaaaagc
3061 agtctcaact aacctggaca accaaggTcc ctgagacact gagccactaa caagtCagcc
3121 tactccagct gttatgaggc ccccaaaaaca tatgcaacat aggattgcct ggtccagcct

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FIG. 7

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3181 cagcaagaga atacacacct aaccacagag agacttcccc aagggattgg ggaggtctgg
3241 ggtttgagaga gttgcgggatt gtcccttgat gattggaagg aggtattgga tgagaatgaa
3301 tcaggggggaa gactaggaag gggataatga tggaactgta aaaaaaaaaa
```

SEQ ID NO:14
Size: 380
PRT FEN1

```
1 MGIQGLAKLI ADVAPSAIRE NDIKSYFGRK VAIDASMSIY QFLIAVRQGG DVLQNEEGET
61 TSHLMGMFYR TIRMMENGIK PVYVFDGKPP QLKSGELAKR SERRAEAEKQ LQQAQAAGAE
121 QEVEKFTKRL VKVTKQHNDK CKHLLSLMGI PYLDAPSEAE ASCAALVKAG KVYAAATEDM
181 DCLTFGSPVL MRHLTASEAK KLPIQEFHLS RILQELGLNQ EQFVDLCILL GSDYCESIRG
241 IGPKRAVDLI QKHKSIEEIV RRLDPNKYPV PENWLHKEAH QLFLEPEVLD PESVELKWSE
301 PNEEELIKFM CGEKQFSEER IRSGVKRLSK SRQGSTQGRL DDFFKVTGSL SSAKRKEPEP
361 KGSTKKKAKT GAAGKFKRGK
```

FIG. 7
(CONT.)

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SEQ ID NO:15
Size: 4276
DNA REV1

```

1 agagccaccg cggagcgcgc gcgggggttg ttgccgcgag cgtggggggag cgtggaccgc
61 ggcgtgctc agcgggtggg ctgccttccc ccggccctcc tccctgggtc ctggcgaggg
121 cactggcggc ggcggggccg gggtccgcga ggccggagaa ggcgcgggg cccgggcatg
181 gtggtctggg gcaacgcgga agaagctcca ccatgaggcg aggtggatgg aggaagcgag
241 ctgaaaatga tggctgggaa acatgggggt ggtatatggc tgccaaggctc cagaaattgg
301 aggaacagtt tcgatcagat gctgctatgc agaaggatgg gacttcatct acaattttta
361 gtggagttgc catctatggt aatggataca cagatccttc cgctgaggaa ttgagaaaac
421 taatgatgtt gcatggaggt caataccatg tatattattc cagatctaaa acaacacata
481 ttattgccac aaatcttccc aatgccaaaa ttaaagaatt aaagggggaa aaagtaattc
541 gaccagaatg gattgtggaa agcatcaaaag ctggacgact cctctcctac attccatata
601 agctgtacac caagcagtc agtgtgcaga aaggtctcag ctttaatect gtatgcagac
661 ctgaggatcc tctgccaggt ccaagcaata tagccaaaca gctcaacaac agggtaaatac
721 acatcgttaa gaagattgaa acggaaaatg aagtcaaagt caatggcatg aacagtggga
781 atgaagaaga tgaaaataat gatttttagtt ttgtggatct ggagcagacc tctccgggaa
841 ggaaacagaa tggaaattccg catcccagag ggagcactgc catttttaaa ggacacactc
901 ctagctctaa tggcgcctta aagacacagg attgcttggg gcccatgggtc aacagtgttg
961 ccagcaggct ttctccagcc ttttcccagg agggaggataa ggctgagaag agcagcactg
1021 atttcagaga ctgcactctg cagcagttgc agcaaagcac cagaaacaca gatgctttgc
1081 ggaatccaca cagaactaat tctttctcat tatcaccttt gcacagtaac actaaaatca
1141 atggtgctca ccactccact gttcaggggc cttcaagcac aaaaagcact tcttcagtat
1201 ctacgttttag caaggcagca ccttcagctgc catccaaacc ttcagactgc aatttttatt
1261 caaacttcta ttctcattca agactgcata acatatcaat gtggaagtgt gaattgactg
1321 agttttgtcaa taccctacaa agacaaagta atggtatctt tccaggaagg gaaaagttaa
1381 aaaaaatgaa aacaggcagg tctgcacttg ttgtaactga cacaggagat atgtcagtat
1441 tgaattctcc cagacatcag agctgtataa tgcatgttga tatggattgc ttctttgtat
1501 cagtgggtat acgaaataga ccagatctca aagggaaaacc agtggctgtt acaagtaaca
1561 gaggcacagg aagggcacct ttacgtcctg gogctáacc ccagctggag tggcagtatt
1621 accagaataa aatcctgaaa ggcaaaagca cagatatacc agattcatca ttgtgggaga
1681 atccagattc tgcgcaagca aatggaattg attctgtttt gtcaagggct gaaattgcat
1741 cttgtagtta tgaggccagg caacttgga ttaagaacgg aatgtttttt gggcatgcta
1801 aacaactatg tcctaattct caagctgttc catacgattt tcatgcatat aagggaagtcg
1861 cacaacatt gtatgaaaca ttggcaagct acactcataa cattgaagct gtcagttgtg
1921 atgaagcgct ggtagacatt accgaaatcc ttgcagagac caaacttact cctgatgaat
1981 ttgcaaatgc tgttcgtatg gaaatcaaag accagacgaa atgtgctgcc tctgttggaa
2041 ttggttctaa tattctcctg gctagaatgg caactagaaa agcaaaacca gatgggcagt
2101 accacctaaa accagaagaa gtagatgatt ttatcagagg ccagctagtg accaatctac
2161 caggagttgg acattcaatg gaatctaagt tggcatcttt gggaattaaa acttgtggag
2221 acttgacgta tatgaccatg gcaaaaactc aaaaagaatt tggccccaaa acaggtcaga
2281 tgctttatag gttctgccgt ggcttgatg atagaccagt tcgaactgaa aaggaaagaa
2341 aatctgtttc agctgagatc aactatggaa taaggtttac tcagccaaa gaggcagaag
2401 cttttcttct gagtctttca gaagaaattc aaagaagact agaagccact ggcattgaagg
2461 gtaaacgtct aactctcaaa atcatggtac gaaagcctgg ggctcctgta gaaactgcaa
2521 aatttgaggg ccatggaatt tgtgataaca ttgccaggac tgtaactctt gaccaggcaa
2581 cagataatgc aaaaataatt ggaaaggcga tgctaaacat gtttcataca atgaaactaa
2641 atatatcaga tatgagaggg gttgggattc acgtgaatca gttggttcca actaatctga
2701 acccttccac atgtcccagt cgcccatcag ttcagtcaag ccactttcct agtgggtcat
2761 actctgtccg tgatgtcttc caagttcaga aagctaagaa atccaccgaa gaggagcaca
2821 aagaagtatt tcgggtgct gtggatctgg aaatatcatc tgcttctaga acttgacctt
2881 tcttgccacc ttttctgca catctgccga ccagtcctga tactaacaag gctgagtctt
2941 cagggaatg gaatgggtct catactcctg tcagtgtgca gtcgagactt aacctgagta
3001 tagagggtccc gtcaccttcc cagctggatc agtctgtttt agaagcactt ccacctgatc
3061 tccgggaaca agtagagcaa gtcgtgtgct tccagcaagc agagtcacat ggcgacaaaa
3121 agaaagaacc agtaaatggc tgtaatacag gaattttgcc acaaccagtt gggacagtct
3181 tgttgcaaat accagaacct caagatacga acagtgcgc aggaataaat ttaatagccc

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FIG. 8

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3241 ttccagcatt ttcacaggtg gaccctgagg tatttgctgc ccttcctgct gaacttcaga
3301 gggagctgaa agcagcgtat gatcaaagac aaaggcaggg cgagaacagc actcaccagc
3361 agtcagccag cgcatctgtg ccaaagaatc ctttacttca tctaaaggca gcagtgaag
3421 aaaagaaaag aaacaagaag aaaaaaacca ttggttcacc aaaaaggatt cagagtcctt
3481 tgaataacaa gctgcttaac agtcctgcaa aaactctgcc aggggcctgt ggcagtcccc
3541 agaagttaat tgatgggttt ctaaaacatg aaggacctcc tgcagagaaa cccctggaag
3601 aactctctgc ttctacttca ggtgtgccag gccttttctag tttgcagtct gaccagctg
3661 gctgtgtgag acctccagca cccaatctag ctggagctgt tgaattcaat gatgtgaaga
3721 ccttgctcag agaatygata actacaattt cagatccaat ggaagaagac atttccaag
3781 ttgtgaaata ctgtactgat ctaatagaag aaaaagattt ggaaaactg gatctagtta
3841 taaaatacat gaaaaggctg atgcagcaat cgggtggaatc ggtttggaa atggcatttg
3901 actttattct tgacaatgtc caggtgggtt tacaacaaac ttatggaagc acattaaag
3961 ttacataaat attaccagag agcctgatgc tctctgatag ctgtgccata agtgcttggtg
4021 aggtatttgc aaagtgcag atagtaatgc tcggagtttt tataatttta aatttctttt
4081 aaagcaagtg ttttgtacat ttcttttcaa aaagtgccaa atttgcagt attgcatgta
4141 aataattgtg ttaattattt tactgtagca tagattctat ttacaaaatg tttgtttata
4201 aagttttatg gattttttaca gtgaagtgtt tacagttggt taataaagaa ctgtatgtaa
4261 aaaaaaaaaa aaaaaa

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SEQ ID NO:16

Size: 1251

PRT REV1

```

1 MRRGGWRKRA ENDGWETWGG YMAAKVQKLE EQFRSDAAMQ KDGTSSSTIFS GVAIYVNGYT
61 DPSAEELRKL MMLHGGQYHV YYSRSKTHI IATNLPNAKI KELKGEKVR PEWIVESIKA
121 GRLLSYIPYQ LYTKQSSVQK GLSFNPVCRP EDPLPGPSNI AKQLNNRVNH IVKKIETENE
181 VKVNGMNSWN EEDENNDFS FVDLEQTS PGR KQNGIPHPRG STAIFNGHTP SSNGALKTQD
241 CLVPMVNSVA SRLSPAFS QE EDKAEKSSTD FRDCTLQQLQ QSTRNTDALR NPHRTNSFSL
301 SPLHSNTKIN GAHHSTVQGP SSTKSTSSVS TFSKAAPSVP SKPSDCNFIS NFYSHSRLHH
361 ISMWKCELTE FVNTLQRQSN GIFFGREKLK KMKTGRSALV VTDTGDM SVL NSPRHQSCIM
421 HVDMD CFFVS VGIRNRPD LK GKPVAVTSNR GTGRAPLRPG ANPQLEWQYY QNKILKGKAA
481 DIPDSSLWEN PDSAQANGID SVLSRAEIAS CSYEARQLGI KNGMFFGHAK QLCPNLQAVP
541 YDFHAYKEVA QTLYETLASY THNIEAVSCD EALVDITEIL AETKLT PDEF ANAVRMEIKD
601 QTKCAASVGI GSNILLARMA TRKAKPDGQY HLKPEEVDDF IRGQLVTNLP GVGHSMESKL
661 ASLGIKTCGD LQYMTMAKLQ KEFGPKTGQM LYRFCRGLDD RPVRTEKERK SVSAEINYGI
721 RFTQPKAEAE FLLSLSEEIQ RRLEATGMKG KRLTLKIMVR KPGAPVETAK FGGHGICDNI
781 ARTVTLDQAT DNAKIIGKAM LNMFHTMKLN ISDMRGVGIH VNQLVPTNLN PSTCPSRPSV
841 QSSHFP SGSY SVRDVFQVQK AKKSTEEHK EVFRAVDLE ISSASRTCTF LPPFPAHLPT
901 SPDTNKAESS GKWNGLHTPV SVQSRLNLSI EVSPSQLDQ SVLEALPPDL REQVEQVCAV
961 QQAESHGDKK KEPVNGCNTG ILPQPVGTVL LQIPEPQESN SDAGINLIAL PAFSQVDPEV
1021 FAALPAELQR ELKAA YDQRQ RQGENSTHQQ SASASVPKNP LLHLKAAVKE KKRNNKKKTI
1081 GSPKRIQSPL NNKLLNSPAK TLPGACGSPQ KLIDGFLKHE GPPAEKPLEE LSASTSGVPG
1141 LSSLQSDPAG CVRPPAPNLA GAVEFNDVKT LLREWITTIS DPMEEDILQV VKYCTDLIEE
1201 KDLEKLDLVI KYMKRLMQQS VESVWNMAFD FILDNVQVVL QQTYGSTLKV T

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FIG. 8
(CONT.)

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SEQ ID NO:17

Size: 2957

DNA APEI

```

1  ctgcagatag cactgggaaa gacaccgcgg aactcccgcg agcgagagccc gccaaaggccc
61  ctccagggac ctgtcttccct aacgtccagg gagcccgagc caactcgcgc cttacattcg
121 tatccgtttt cctatctctt tcccgtgggc agcccagcct tctccactgt ttttttcctc
181 ttgcacagag ttagaatctt aagtcagtggt cacacaatgt gctgtgcatc tggcacaacg
241 ataaacagcc gagggagggt tggggactaa gtgcctagag aattagagga gggaggcgag
301 gctaagcgtc cgtcacgtgg tgtcagacag accaatcacg cgcattcttc ggccacgaca
361 agcgcgccct tgatcacgtg accagggtccg ctaccacgtg gggggctcag cgtgcaccct
421 tctttgtgct cgggttagga ggagctaggc tgccatcggy ccggtgcaga tacgggggtg
481 ctcttttctc cataagaggg gcttcgctgg cagtctgaac ggcaagcttg agtcaggacc
541 cttaattaa atcctcaatt ggctggaggg cagatctcgc gagtagggta caaggcacta
601 tgaaatgatc tagtttcgtg ggtgaggggc tgaaggccct atgatgcacg gagggcgagg
661 aaggatttag agataacgtg gtttaaaggc gggacctggt gcggggacgc tccttgggag
721 gagtcttctc ccagccttag ctggtttcat gatttctttg cgtctgtagg caacgcggta
781 aaaatattgc ttcggtgggt gacgcggtac agctgcccaa gggcgctcgt aacgggaatg
841 ccgaagcggt ggaaaaaggg agcgggtggc gaagacgggg atgagctcag gacaggtaag
901 ggaatgaaat cagcccttct tcctagaagc tgcggcgggg gtgtttgtca ttccttgat
961 gtacggtaag tacgggccga ctcatTTTTT caggggtttg tgaagaagtc gcaggaaccg
1021 taggctttcg ttgggtctat agttaacgcc ggatcgcagt tggaaaccac cagctttttg
1081 tcagtatata ttactcattt tatagagcca gaggccaaga agagtaagac ggcgcgaaag
1141 aaaaatgaca aagaggcagc aggagagggc ccagccctgt atgaggaccc ccagatcag
1201 aaaacctcac ccagtggcaa acctgccaca ctcaagatct gctcttgga tgtggatggg
1261 cttcgagcct ggattaagaa gaaaggatta gatgtgagt gaatttgagg gaaagagaca
1321 ttttttagta ttgaatggtc ttagggttta gtcacccctt ttctcgttt agccttcagg
1381 ctgttttatt tttctcctgc ccgtagtttt ctgtggggct tccccagttc tgccagttgt
1441 atttctctaa tgtctgttcc ttactttcca ttgccatttt cttttttagt gttctctcct
1501 ctccccagaa tgttgcaaaa acctcttcac tatacttctt ccattttatc ttctcgtatt
1561 gcattccata tgaagcatgt cctccattcc attaacata gcttaaaatc tttagcttgct
1621 atccactgcc tatagaaaaa acacatctcc ttggcatagc atgtaagact ttcttacctc
1681 tctatatttg ttttcattta tctagcttag aattgtttga atattgtgct gcttgactcg
1741 aactccttag gccaaagagac tgtttaaccc gtgcgtatct atgacttagc atatagatta
1801 ttcaataaat gttctgctga attgataata cgttttccac ctttcttttc acttacagtg
1861 ggttaaggaa gaagccccag atatactgtg ccttcaagag accaaatggt cagagaacaa
1921 actaccagct gaacttcagg agctgcctgg actctctcat caatactggt cagctccttc
1981 ggacaaggaa ggttacagtg gcgtgggcct gctttccgc cagtgccac tcaaagtttc
2041 ttacggcata ggtgagaccc tattgatgcc taatgcctga actcttcaaa accaattgct
2101 aattctctat ctctgcccc cctcttgatt gctttccctt ttcttatagt tttttatgct
2161 aattctgttt catttctata ggcatgagg agcatgatca ggaaggccgg gtgattgtgg
2221 ctgaatttga ctggtttgtg ctggttaacg catatgtacc taatgcaggc cgaggtctgg
2281 tacgactgga gtaccggcag cgtgggagat aagcctttcg caagtctctg aagggcctgg
2341 ctccccgaaa gcccttgtg ctgtgtggag acctcaatgt ggcacatgaa gaaattgacc
2401 ttcgcaaccc caaggggaac aaaaagaatg ctggcttcac gccacaagag cgccaaggct
2461 tcgggggaatt actgcaggct gtgccactgg ctgacagctt taggcacctc taccccaaca
2521 caccctatgc ctacaccttt tggacttata tgatgaatgc tcgatccaag aatgttggtt
2581 ggcgccctga ttactttttg ttgtccact ctctgttacc tgcattgtgt gacagcaaga
2641 tccgtttcaa ggcctcggc agtgatcact gtccatcac cctataccta gactgtgac
2701 accacccta aatcactttg agcctgggaa ataagcccc tcaactacca ttcttctttt
2761 aaacactctt cagagaaatc tgcattctat ttctcatgta taaaactagg aatcctccaa
2821 ccaggctcct gtgatagagt tcttttaagc ccaagatttt ttatttgagg gttttttgtt
2881 ttttaaaaaa aaattgaaca aagactacta atgactttgt ttgaattatc cacatgaaaa
2941 taaagagcca tagtttc

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FIG. 9

SUBSTITUTE SHEET (RULE 26)

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SEQ ID NO:18
Size: 318
PRT APE1

1	MPKRGKKGAV	AEDGDELRTE	PEAKKSKTAA	KKNDKEAAGE	GPALYEDPPD	QKTSPSGKPA
61	TLKICSWNVD	GLRAWIKKKG	LDWVKEEAPD	ILCLQETKCS	ENKLPAELQE	LPGLSHQYWS
121	APSDKEGYSG	VGLLSRQCPL	KVSYGIGDEE	HDQEGRVIVA	EFDSFVLVTA	YVPNAGRGLV
181	RLEYRQRWDE	AFRKFLKGLA	SRKPLVLCGD	LNVAHEEIDL	RNPKGNGKNA	GFTPQEAQGF
241	GELLQAVPLA	DSFRHLYPNT	PYAYTFWTYM	MNARSKNVGW	RLDYFLLSHS	LLPALCDSKI
301	RSKALGSDHC	PITLYLAL				

FIG. 9
(CONT.)

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SEQ ID NO:19
Size: 1161
DNA CDK3

```

1 ccacatggaa gctggaggag caaccgggag cgctgggctg ggggtgcaaat tgcccagtgc
61 cttctgtttc ccaggcagct ctgtggccat ggatatgttc cagaaggtag agaagatcgg
121 agagggcacc tatggggtgg tgtacaaggc caagaacagg gagacagggc agctgggtggc
181 cctgaagaag atcagactgg atttggagat ggaggggggc ccaagcactg ccatcagggga
241 gatctcgctg ctcaaggaac tgaagcacc ccaacatcgtc cgactgctgg acgtgggtgca
301 caacgagagg aagctctatc tgggtgtttga gttcctcagc caggacctga agaagtacat
361 ggactccacc ccaggctcag agctccccct gcacctcacc aagagctacc tcttccagct
421 gctgcagggg gtgagtttct gccactcaca tcgggtcacc caccgagacc tgaagcccca
481 gaacctgctc atcaatgagt tgggtgccat caagctggct gacttcggcc tggctcgcg
541 cttcgggggtg cccctgcgca cctacaccca tgaggtgggtg acactgtggt atcgcgcccc
601 cgagattctc ttgggcagca agttctatac cacagctgtg gatattctgga gcattgggtg
661 catctttgca gagatggtga ctcgaaaagc cctgtttcct ggtgactctg agattgacca
721 gctctttcgt atctttcgta tgctggggac acccagcgaa gacacatggc ccgggggtcac
781 ccagctgcct gactataagg gcagcttccc taagtggacc aggaagggac tgggaagagat
841 tgtgccaat ctggagccag agggcagggga cctgctcatg caactcctgc agtatgaccc
901 cagccagcgg atcacagcca agactgccct ggcccacccg tacttctcat cccctgagcc
961 ctccccagct gcccgccagt atgtgctgca gcgattccgc cattgagaat gtcaaggcca
1021 cactcagatc ctttctcgag cagcagctgc tgccccagct gcctcctacc cattgccaa
1081 agaggatgca tctggggaga gcaaagcact aaggaattca gcatcagcct gcagagggct
1141 gagtctgggt tagtcctgcc c

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SEQ ID NO:20
Size: 305
PRT CDK3

```

1 MDMFQKVEKI GEGTYGVVYK AKNRETGQLV ALKKIRLDLE MEGVPSTAIR EISLLKELKH
61 PNIVRLLDV HNERKLYLVF EFLSQDLKKY MDSTPGSELP LHLIKSYLFQ LLQGVSFCHS
121 HRVIHRDLKP QNLLINELGA IKLADFLAR AFGVPLRTYT HEVVTLWYRA PEILLGSKFY
181 TTAVDIWSIG CIFAEMVTRK ALFPGDSEID QLFRIFRMLG TPSEDWPGV TQLPDYKGSF
241 PKWTIRGLEE IVPNLEPEGR DLLMQLLQYD PSQRITAKTA LAHPYFSSPE PSPAARQYVL
301 QRFRRH

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FIG. 10

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SEQ ID NO:21
Size: 2297
DNA PIMI

```

1  gcgcgcgcac  ctggaggttg  ggatgctctt  gtccaaaatc  aactcgcttg  cccacctgcy
61  cgcgcgcgcc  tgcaacgacc  tgcacgccac  caagctggcg  ccgggcaagg  agaaggagcc
121 cctggagtcg  cagtaccagg  tgggcccgc  actgggcagc  ggcggttcg  gctcggctta
181 ctcaggcatc  cgcgtctccg  acaacttgcc  ggtggccatc  aaacacgtgg  agaaggaccg
241 gatttccgac  tggggagagc  tgcctaattg  cactcgagt  cccatggaag  tggctcctgt
301 gaagaagggt  agctcgggtt  tctccggcgt  cattaggctc  ctggactgg  tgcgagggcc
361 cgacagtttc  gtcctgatcc  tggagaggcc  cgagccgggt  caagatctct  tcgacttcat
421 cagggaagg  ggagccctgc  aagaggagct  ggcccgcagc  ttcttctggc  aggtgctgga
481 ggccgtgcgg  cactgccaca  actgcggggt  gctccaccgc  gacatcaagg  acgaaaacat
541 ccttatcgac  ctcaatcgcg  gcgagctcaa  gctcatcgac  ttccgggtcg  ggcgctgct
601 caaggacacc  gtctacacgg  acttcgatgg  gaccgcagtg  tatagccctc  cagagtggat
661 ccgctaccat  cgctaccatg  gcaggtcggc  ggcagtctgg  tccctgggga  tcctgctgta
721 tgatatgggt  tgtggagata  ttcccttoga  gcatgacgaa  gagatcatca  ggggccagggt
781 tttcttcagg  cagaggggtc  cttcagaatg  tcagcatctc  attagatgg  gcttggccct
841 gagaccatca  gataggccaa  ccttcgaaga  aatccagaac  catccatgga  tgcaagatgt
901 tctcctgccc  caggaaactg  ctgagatcca  cctccacagc  ctgtcgccgg  ggcccagcaa
961 atagcagcct  ttctggcagg  tcctccctc  tcttgtcaga  tgcccaggag  ggaagcttct
1021 gtctccagct  ttcccagta  ccagtgcac  gtctcgccaa  gcaggacagt  gcttgataca
1081 ggaacaacat  ttacaactca  tccagatcc  caggcccctg  gaggtgcct  cccaacagt
1141 gggaagagt  actctccagg  ggtccctagg  ctcaactcct  cccatagata  ctctcttctt
1201 ctcataggt  tccagcattg  ctggactctg  aaatatccc  ggggtggggg  gtgggggttg
1261 gtcagaacc  tgccatggaa  ctgtttcctt  catcatgagt  tctgtgta  gccgcgatgg
1321 gtcaggtagg  ggggaaacag  gttgggatgg  gataggacta  gcaccatttt  aagtcctgt
1381 cactcttcc  gactctttct  gagtgccttc  tgtggggact  ccgctgtgc  tgggagaaat
1441 acttgaactt  gcctctttta  cctgctgctt  ctccaaaaat  ctgcctgggt  tttgttccct
1501 atttttctct  cctgtcctcc  ctcacccct  ccttcatatg  aaaggtgcca  tggagaggcc
1561 tacaggcca  aacgctgagc  cactgcctt  ttttctcct  cctttagtaa  aactccgagt
1621 gaactggtct  tcttttttgg  tttttactta  actgtttcaa  agccaagacc  tcacacacac
1681 aaaaaatgca  caaacaatgc  aatcaacaga  aaagctgtaa  atgtgtgtac  agttggcatg
1741 gtagtataca  aaaagattgt  agtggatcta  atttttaaga  aattttgcct  ttaagttatt
1801 ttacctgttt  ttgtttcttg  ttttgaaaga  tgcgcattct  aacctggagg  tcaatgttat
1861 gtattttatt  atttatttat  ttggttccct  tcctannnnn  nnnnnngctg  ctgccctagt
1921 tttctttcct  cctttcctcc  tctgacttgg  ggaccttttg  ggggaggggt  gcgacgcttg
1981 ctcgtttgt  ggggtgacgg  gactcaggcg  ggacagtgt  gcagctccct  ggcttctgtg
2041 gggccctca  cctacttacc  caggtgggtc  ccggtctgt  ggggtgatgg  gaggggcatt
2101 gctgactgtg  tatataggat  aattatgaaa  agcagttctg  gatggtgtgc  cttccagatc
2161 ctctctgggg  ctgtgttttg  agcagcaggt  agcctgctgg  ttttatctga  gtgaaatact
2221 gtacagggga  ataaaagaga  tcttattttt  ttttttatac  ttggcgcttt  ttgaataaaa
2281 accttttgtc  ttaaaac

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SEQ ID NO:22
Size: 313
PRT PIMI

```

1  MLLSKINSLA  HLRARACNDL  HATKLAPGKE  KEPLESQYQV  GPLLGSGGFG  SVYSGIRVSD
61  NLPVAIKHVE  KDRISDWGEL  PNGTRVPMVE  VLLKKVSSGF  SGVIRLLDWF  ERPDFVLIL
121 ERPEPVQDLF  DFITERGALQ  EELARSFFWQ  VLEAVRHCHN  CGVLHRDIKD  ENILIDLNRG
181 ELKLIDFGSG  ALLKDTVYTD  FDGTRVYSPP  EWIRYHRYHG  RSAAVWSLGI  LLYDMVCGDI
241 PFEHDEEIR  GQVFFRQRVS  SECQHLIRWC  LALRPSDRPT  FEEIQNHPPM  QDVLLPQETA
301 EIHLSLSPG  PSK

```

FIG. 11

SEQ ID NO:23
Size: 3178
DNA CDC7L1

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1 gatctcttgg agacggcgac ccaggcatct ggggagccac agaagtcgta ctcccttaaa
61 ccctgctttg ctccccctgt ggatgtaacc ccttagctgg cattttgcat ctcaattggc
121 ttgtgatgga ggcgctcttg gggattcaga tggatgagcc aatggctttt tctccccagc
181 gtgaccgggt tcaggctgaa ggctctttaa aaaaaaacga gcagaatttt aaacttgcag
241 gtgttaaaaa agatattgag aagctttatg aagctgtacc acagcttagt aatgtgttta
301 agattgagga caaaattgga gaaggcactt tcagctctgt ttatttggcc acagcacagt
361 tacaagtagg acctgaagag aaaattgctc taaaacactt gattccaaca agtcatccta
421 taagaattgc agctgaactt cagtgcctaa cagtggctgg ggggcaagat aatgtcatgg
481 gagttaaata ctgctttagg aagaatgata atgtagttaa tgctatgcca tatctggagc
541 atgagtcgtt tttggacatt ctgaattctc tttcctttca agaagtacgg gaatatatgc
601 ttaatctgtt caaagctttg aaacgcattc atcagtttgg tattgttcac cgtgatgtta
661 agcccagcaa ttttttatat aataggcgcc tgaaaaagta tgccttggtg gactttgggt
721 tggcccaagg aacccatgat acgaaaatag agcttcttaa atttgtccag tctgaagctc
781 agcaggaaag gtgttcacaa aacaaatccc acataatcac aggaaacaag attccactga
841 gtggcccagt acctaaaggag ctggatcagc agtccaccac aaaagcttct gttaaaagac
901 cctacacaaa tgcacaaatt cagattaaac aaggaaaaga cggaaaggag ggtatctgtg
961 gcctttctgt ccagcgtctc gtttttggag aaagaaattt caatatacac agctccattt
1021 cacatgagag ccctgcagtg aaactcatga agcagtcaaa gactgtggat gtactgtcta
1081 gaaagttagc aacaaaaaag aaggctatct ctacgaaagt tatgaatagt gctgtgatga
1141 ggaaaactgc cagttcttgc ccagctagcc tgacctgtga ctgctatgca acagataaag
1201 tttgtagtat ttgcctttca aggcgtcagc aggttgcccc tagggcaggt acaccaggat
1261 tcagagcacc agaggtcttg acaaagtgcc ccaatcaaac tacagcaatt gacatgtggt
1321 ctgcagggtg catatttctt tctttgttta gtggacgata tccattttat aaagcaagtg
1381 atgatttaac tgctttggcc caaattatga caattagggg atccagagaa actatccaag
1441 ctgctaaaac ttttgggaaa tcaatattat gtagcaaaga agttccagca caagacttga
1501 gaaaactctg tgagagactc aggggtatgg attctagcac tcccaagtta acaagtgata
1561 tacaagggca tgcttctcat caaccagcta tttcagagaa gactgaccat aaagcttctt
1621 gcctcgttca aacacctcca ggacaatact cagggaattc atttaaaaag ggggatagta
1681 atagctgtga gcattgtttt gatgagtata ataccaattt agaaggttgg aatgaggtag
1741 ctgatgaagc ttatgacctg cttgataaac ttctagatct aaatccagct tcaagaataa
1801 cagcagaaga agctttgttg catccatttt ttaaagatat gagcttgtga taatggatct
1861 tcattttaatg tttactgtta tgaggtagaa taaaaaagaa tactttgtaa tagccacaag
1921 ttcttgttta gagaccagag caggattaat aatttatttt aacatttttag tgtttggtgg
1981 cacattctaa aatatagatt aagaatactt aaaatgcctg ggatagttct tgggactaac
2041 aacatgatct tctttgagtt aaacctacct aagtagattt taggtgggtt cctattaggt
2101 cagattttta gcttccctaa ttacctttca ctgacataca gaaaaaggag cagtttttagt
2161 tttaattaat taaaattaac agatgtgatg aggattaaat gaatcaaaag acttaatttg
2221 tagattcttt tagagttatg agctaggtat agtttgggga aactcaacct ggtgctgggtg
2281 ctcttaacaa ttttgtaaat aaagaagata atttcctttt ctagaggtag atattaggcc
2341 ttttatgaac actaaaacaa tgaggaaatg ttggtcatgg ggcaaagtat cacttaaaat
2401 tgaattcatc cattttttaa aaacacttca tgaaagcatt ctggtgtgaa ttgccatttt
2461 tttcttactg gcttctcaat tttcttctct ctctgcccct acctaaaaca ttctctctcg
2521 aaattacatg gtgctgacca caaagtttct ggatgtttta ttaaattattg tacgtgttta
2581 cagttgggaa tttaaaataa tacatacact ggttgataaa gggagctgc aggaccaagg
2641 tgaagattga tagtccaaat gcttttcttt tttgagttgt atattttttc acaccatctt
2701 agatataatt aggtagctgc tgaaaggaaa agtgaataca gaattgacgg tattatttga
2761 gatttttctt ctgcgtagag ccattccagat ctctgtatcc tgttttgact aagctctagg
2821 tgggttggga agacagataa tgaagttagc aaagagaaaa ggacccaaga tagaggttta
2881 tattcagaaa tggatatatat caatgacagc atatcaaact tcctatggga aaaagtctgg
2941 tgggtggtca gctgacagat ttcccattta gtagtcatag aatacagaaa tagtttaggg
3001 acatgtattc attttgttat tttagcattt gataggtcag tatatctacc taatctgttt
3061 ggtaagtata ggatatataa accattacca ttgatctgtc ttatgccata atcttaaaaa
3121 aaaattgaat gctcttgaat ttgtatatcc aataaagtta tctttttata aaaaaaaa

```

FIG. 12

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SEQ ID NO:24
Size: 574
PRT CDC7L1

1	MEASLGIQMD	EPMAFSPQRD	RFOAEGSLKK	NEQNFKLAGV	KKDIEKLYEA	VPQLSNVFKI
61	EDKIGEGTFS	SVYLATAQLQ	VGPEEKIALK	HLIPTSHPIR	IAAELQCLTV	AGGQDNVMGV
121	KYCFRKNDHV	VIAMPYLEHE	SFLDILNSLS	FQEVREYMLN	LFKALKRIHQ	FGIVHRDVKP
181	SNFLYNRRLK	KYALVDFGLA	QGTHDTKIEL	LKFVQSEAQQ	ERCSQNKSHI	ITGNKIPLSG
241	PVPKELDQQS	TTKASVKRPY	TNAQIQIKQG	KDGKEGSVGL	SVQRSVFGER	NFNIHSSISH
301	ESPAVKLMKQ	SKTVDVLSRK	LATKKKAIST	KVMNSAVMRK	TASSCPASLT	CDCYATDKVC
361	SICLSRRQQV	APRAGTPGFR	APEVLTKCPN	QTTAIDMWSA	GVIFLSLLSG	RYPFYKASDD
421	LTALAQIMTI	RGSRETIQAA	KTFGKSILCS	KEVPAQDLRK	LCERLRGMDS	STPKLTSDIQ
481	GHASHQPAIS	EKTDHKASCL	VQTPPGQYSG	NSFKKGDSNS	CEHCFDEYNT	NLEGWNEVPD
541	EAYDLLDKLL	DLNPASRITA	EEALLHPFFK	DMSL		

FIG. 12
(CONT.)

21/84

SEQ ID NO:25
Size: 1427
DNA CDK7

```

1  tgggtgttgg  aggctttaag  gtagctttaa  attcgtgttg  tcctgggagc  tcgccctttt
61  cggctggagt  cgggctttac  ggcgcgggat  ggctctggac  gtgaagtctc  gggcaaagcg
121  ttatgagaag  ctggacttcc  ttggggaggg  acagtttgcc  accgtttaca  aggccagaga
181  taagaatacc  aaccaaattg  tcgccattaa  gaaaatcaaa  cttggacata  gatcagaagc
241  taaagatggg  ataaatagaa  ccgccttaag  agagataaaa  ttattacagg  agctaagtca
301  tccaaatata  attggtctcc  ttgatgcttt  tggacataaa  tctaataatta  gccttgtctt
361  tgattttatg  gaaactgatc  tagaggttat  aataaaggat  aatagtcttg  tgctgacacc
421  atcacacatc  aaagcctaca  tgttgatgac  tcttcaagga  ttagaatatt  tacatcaaca
481  ttggatccta  catagggatc  tgaaaccaa  caacttggtg  ctagatgaaa  atggagtctt
541  aaaactggca  gattttggcc  tggccaaatc  ttttgggagc  cccaatagag  cttatacaca
601  tcaggttgta  accagggtgg  atcgggcccc  cgagttacta  tttggagcta  ggatgtatgg
661  tgtaggtgtg  gacatgtggg  ctgttggtcg  tatattagca  gagttacttc  taagggttcc
721  ttttttgcca  ggagattcag  accttgatca  gctaacaaga  atatttgaaa  ctttgggcac
781  accaactgag  gaacagtggc  cggacatgtg  tagtcttcca  gattatgtga  catttaagag
841  tttccctgga  atacctttgc  atcacatctt  cagtgcagca  ggagacgact  tactagatct
901  catacaaggc  ttattcttat  ttaatccatg  tgctcgaatt  acggccacac  aggcactgaa
961  aatgaagtat  ttcagtaatc  ggccagggcc  aacacctgga  tgctcagctgc  caagacccaa
1021  ctgtccagtg  gaaaccttaa  aggagcaatc  aaatccagct  ttggcaataa  aaaggaaaag
1081  aacagaggcc  ttagaacaag  gaggattgcc  caagaaacta  attttttaaa  gagaacactg
1141  gacaacattt  tactactgag  ggaaatagcc  aaaaaggcaa  ataatggaaa  aatagtaaac
1201  attaagtaaa  tgctgtagaa  gtgagtttgt  aaatattcta  cacatgtaaa  atatgtaaaa
1261  ctatgggtta  tttttattaa  atgtatttta  aaataaaaaa  ttaattctgg  tttttctgat
1321  tagagtccca  aagtgagaaa  agttcaatac  tcttgaaatg  tagaattgaa  aatgcattag
1381  ggaaaactta  ataaaaatta  ttaccagtta  tttggaaaaa  aaaaaaa

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SEQ ID NO:26
Size: 346
PRT CDK7

```

1  MALDVKSRK  RYEKLDLGE  GQFATVYKAR  DKNTNQIVAI  KKIKLGRHSE  AKDGINRTAL
61  REIKLLQELS  HPNIIIGLLDA  FGKSNISLV  FDFMETDLEV  IIKDNSLVLT  PSHIKAYMLM
121  TLQGLEYLHQ  HWILHRDLKP  NNLLLDENG  LKLDLFLAK  SFGSPNRAYT  HQVVTRWYRA
181  PELLFGARMY  GVGVDMMWAVG  CILAEALLRV  PFLPGDSDL  QLTRIFETLG  TPTEEQWPD
241  CSLPDYVTFK  SFPGIPLHHI  FSAAGDDL  LIQGLFLFNP  CARITATQAL  KMKYFSNRPG
301  PTPGCQLPRP  NCPVETLKEQ  SNPALAIK  RTEALEQGGL  PKKLIF

```

FIG. 13

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SEQ ID NO:27
Size: 2169
DNA CNK

```

1  ccgcctccga  gtgccttgcg  cggacctgag  ctggagatgc  tggccggggt  accgacgtca
61  gaccccgggc  gcctcatcac  ggaccgcgcg  agcggccgca  cctacctcaa  aggccgcttg
121  ttgggcaagg  ggggcttcgc  ccgctgctac  gaggccactg  acacagagac  tggcagcgcc
181  tacgctgtca  aagtcattcc  gcagagccgc  gtcgccaagc  cgcattcagc  cgagaagatc
241  ctaaatgaga  ttgagctgca  ccgagacctg  cagcaccgcc  acatcgtgcg  tttttcgcac
301  cactttgagg  acgctgacaa  catctacatt  ttcttggagc  tctgcagccg  aaagtccctg
361  gccacatct  ggaaggcccg  gcacaccctg  ttggagccag  aagtgcgcta  ctacctgcgg
421  cagatccttt  ctggcctcaa  gtacttgcac  cagcgcggca  tcttgaccg  ggacctcaag
481  ttgggaaatt  ttttcatcac  tgagaacatg  gaactgaagg  tgggggattt  tgggctggca
541  gcccggttg  agcctccgga  gcagaggaag  aagaccatct  gtggcacc  caactatgtg
601  gctccagaag  tgctgctgag  acagggccac  ggccctgaag  cggatgtatg  gtcactgggc
661  tgtgtcatgt  acacgctgct  ctgcgggagc  cctcccttg  agacggctga  cctgaaggag
721  acgtaccgct  gcatcaagca  ggttcactac  acgctgcctg  ccagcctctc  actgcctgcc
781  cggcagctcc  tggccgccat  ctttcgggcc  tcaccccgag  accgcccctc  tattgaccag
841  atcctgcgcc  atgacttctt  taccaagggc  tacaccccg  atcgactccc  tatcagcagc
901  tgcgtgacag  tcccagacct  gacaccccc  aaccagcta  ggagtctgtt  tgccaaagtt
961  accaagagcc  tctttggcag  aaagaagaag  agtaagaatc  atgccaggga  gagggatgag
1021  gtctccggtt  tggtagcg  cctcatgcgc  acatccgttg  gccatcagga  tggcaggcca
1081  gaggctccag  cagcttctgg  cccagccct  gtcagcctgg  tagagacagc  acctgaagac
1141  agctcacc  gtgggacact  ggcaagcagt  ggagatggat  ttgaagaagg  tctgactgtg
1201  gccacagtag  tggagtcagc  cttttgtgct  ctgagaaatt  gtatagcttt  catgccccca
1261  gcggaacaga  acccgcccc  cctggcccg  ccagagcctc  tgggtgtggg  cagcaagtgg
1321  gttgactact  ccaataagtt  cggctttggg  tatcaactgt  ccagccgcg  tgtggctgtg
1381  ctcttcaacg  atggcacaca  tatggccctg  tcggccaaca  gaaagactgt  gcaataaat
1441  cccaccagca  caaagcactt  ctcttctcc  gtgggtgctg  tgccccgggc  cctgcagcct
1501  cagctgggta  tcttgoggta  cttgcctcc  tacatggagc  agcacctcat  gaaggggtgga
1561  gatctgcca  gtgtggaaga  ggtagaggta  cctgctccgc  ccttgcctgt  gcagtgggtc
1621  aagacggatc  aggtctctct  catgctgttt  agtgatggca  ctgtccaggt  gaacttctac
1681  ggggaccaca  ccaagctgat  tctcagtggc  tgggagcccc  tccttgtgac  ttttgtggcc
1741  cgaaatcgta  gtgcttgtac  ttacctgct  tccaccttc  ggcagctggg  ctgctctcca
1801  gacctgcggc  agcgactccg  ctatgctctg  cgctgctcc  gggaccgag  cccagcttag
1861  gacccaagcc  ctgaaggcct  gaggcctgtg  cctgtcaggc  tctggccctt  gcctttgtgg
1921  ccttccccct  tcctttgggt  cctcactggg  ggctttgggc  cgaatcccc  agggaatcag
1981  ggaccagctt  tactggagtt  gggggcggt  tgtcttcgct  ggctcctacc  ccatctccaa
2041  gataagcctg  agccttagct  cccagctagg  gggcgttatt  tatggaccac  ttttatttat
2101  tgtcagacac  ttatttattg  ggatgtgagc  cccagggggc  ctctcctag  gataataaac
2161  aattttgca

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SEQ ID NO:28
Size: 607
PRT CNK

```

1  MLAGLPTSDP  GRLITDPRSG  RTYLKGRLLG  KGGFARCYEA  TDTETGSAYA  VKVIPQSRVA
61  KPHQREKILN  EIELHRDLQH  RHIVRFSSHFF  EDADNIYIFL  ELCSRKSLAH  IWKARHTLLE
121  PEVRYYLRLQI  LSGLYLHQR  GILHRDLKLG  NFFITENMEL  KVGDFGLAAR  LEPPEQRKKT
181  ICGTPNYVAP  EVLLRQGHGP  EADVWSLGCV  MYTLGCSPP  FETADLKETY  RCIKQVHYTL
241  PASLSLPAEQ  LLAAILRASP  RDRPSIDQIL  RHDFFTKGYT  PDRLPISSCV  TVPDLTPPNP
301  ARSLFAKVTK  SLFGRKKKSK  NHAQERDEVS  GLVSGLMRTS  VGHQDARPEA  PAASGPAPVS
361  LVETAPEDSS  PRGTLASSGD  GFEEGLTVAT  VVESALCALR  NCIAFMPPAE  QNPAPLAQPE
421  PLVWVSKWVD  YSNKFEGFYQ  LSSRRVAVLF  NDGTHMALSA  NRKTVHYNPT  STKHFSFSVG
481  AVPRALQPQL  GILRYFASYM  EQHLMKGGDL  PSVEEVEVPA  PPLLLQWVKT  DQALLMLFSD
541  GTVQVNFYGD  HTKLILSGWE  PLLVTFVARN  RSACTYLASH  LRQLGCSDDL  RQRLRYALRL
601  LRDRSPA

```

FIG. 14

SUBSTITUTE SHEET (RULE 26)

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SEQ ID NO:29
Size: 1321
DNA PRL-3

```
1  tgactatcca gctctgagag acgggagttt ggagttgccc gctttacttt ggttgggttg
61  gggggggcgg cgggctgttt tgttcctttt cttttttaag agttgggttt tcttttttaa
121 ttatccaaac agtgggcage ttcctccccc acaccaagt atttgacaa tatttgtgcg
181 gggatatggg gtgggttttt aaatctcgtt tctcttgga aagcacagg atctcgttct
241 cctcattttt tgggggtgtg tggggacttc tcaggctgtg tccccagct tctctgcagt
301 cccttctgcc ctgccgggcc cgtcgggagg cgccatggct cggatgaacc gcccgggccc
361 ggtggaggtg agctacaaac acatgcgctt cctcatcacc cacaaccca ccaacgccac
421 gctcagcacc ttcattgagg acctgaagaa gtacggggct accactgtgg tgcgtgtgtg
481 tgaagtgacc tatgacaaaa cgccgctgga gaaggatggc atcaccttg tggactggcc
541 gtttgacgat ggggcgcccc cgcccgcaa ggtagtggaa gactggctga gcctggtgaa
601 ggccaagttc tgtgaggccc cggcagctg cgtggctgtg cactgcgtgg cgggcctggg
661 ccggaagcgc cgcgagcca tcaacagcaa gcagctcacc tacctggaga aataccggcc
721 caaacagagg ctgcggttca aagaccaca cagcacaag acccgtgct gcgttatgta
781 gctcaggacc ttggctgggc ctggtcgtca tgtaggtcag gaccttggct ggacctggag
841 gccctgcccc gccctgctct gccagcccc gcaggggctc caggccttg ctggcccccac
901 atcgctttt cctccccgac acctcgtgc acttgtgtcc gaggagcgag gagcccctcg
961 ggccctgggt ggcctctggg cctttctcc tgtctccgc actccctctg gcggcgctgg
1021 ccgtggctct gtctctctga ggtgggtcgg gcgcccctct cccgccccct cccacaccag
1081 ccaggctggt ctctcttagc ctgttgttg tggggtgggg gtatatattg taaccactgg
1141 gccccagcc cctcttttgc gacccttgt cctgacctgt tctcgccacc ttaaattatt
1201 agaccccggt gcagtcaggt gctccggaca cccgaaggca ataaaacagg agccgtgaaa
1261 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
1321 a
```

SEQ ID NO:30
Size: 148
PRT PRL-3

```
1  MARMNRPAPV EVSYKHMRF ITHNPTNATL STFIEDLK KY GATTVVVRCE VTYDKTFLEK
61  DGITVVDWPF DDGAPPPGV VEDWLSLVKA KFCEAPGSCV AVHCVAGLGR KRRGAINSKQ
121 LTYLEKYRPK QRLRFKDPHT HKTRCCVM
```

FIG. 15

24/84

SEQ ID NO:31
Size: 3696
DNA STK2 (NEK4)

```

1  ggatcgctat  ggcagcggcg  tcgtcgcggg  ccggggcccca  gcaatcccgc  ccggggcccg
61  ctgcctcaac  agccgcccc  actgccccct  ctcgggcatg  aaccgagctt  cttgttgccg
121  cccgctgccc  tacccgccc  tgccgcccga  tcccgaactt  gggccagcgc  tgggaacatg
181  cccctggccg  cctactgcta  cctgcgggtc  gtgggcaagg  ggagctatgg  agaggtgacg
241  cttgtgaagc  accggcggga  cggcaagcag  tatgtcatca  aaaaactgaa  cctccgaaat
301  gcctctagcc  gagagcggcg  agctgctgaa  caggaagccc  agctcttgct  tcagttgaag
361  catcccaaca  ttgtcaccta  caaggagtca  tgggaaggag  gagatggtct  gctctacatt
421  gtcatgggct  tctgtgaagg  aggtgatttg  taccgaaagc  tcaaggagca  gaaagggcag
481  cttctgcctg  agaatcaggt  ggtagagtgg  tttgtacaga  tcgccatggc  tttgcagtat
541  ttacatgaaa  aacacatcct  tcatcgagat  ctgaaaactc  aaaatgtctt  cctaacaaga
601  acaaacatca  tcaaagtagg  ggacctagga  attgcccag  tgtagagaa  ccactgtgac
661  atggctagca  ccctcattgg  cacaccctac  tacatgagcc  ctgaattgtt  ctcaaacaaa
721  ccctacaact  ataagtctga  tgtttgggct  ctaggatgct  gtgtctatga  aatggccacc
781  ttgaagcatg  ctttcaatgc  aaaagatatg  aattctttag  ttatcggat  tattgaagga
841  aagctgccac  caatgccaag  agattacagc  ccagagctgg  cagaactgat  aagaacaatg
901  ctgagcaaaa  ggctgaaga  aaggcgtct  gtgaggagca  tcttgaggca  gccttatata
961  aagcggcaaa  tctccttctt  tttggaggcc  acaaagataa  aaacctccaa  aaataacatt
1021  aaaaatggtg  actctcaatc  caagcctttt  gctacagtgg  tttctggaga  ggcagaatca
1081  aatcatgaag  taatccaccc  ccaaccactc  tcttctgagg  gctcccagac  atatataatg
1141  ggtgaaggca  aatgtttgtc  ccaggagaaa  cccaggccct  ctggtctctt  gaagtcaact
1201  gccagtctga  aagcccatac  ctgcaaacag  gacttgagca  ataccacaga  actagccaca
1261  atcagtagcg  taaatattga  catcttacct  gcaaaaggga  gggattcagt  gagtgtggc
1321  tttgttcagg  agaatcagcc  aagatatttg  gatgcctcta  atgagttagg  aggtatatgc
1381  agtattttct  aagtggaaag  ggagatgctg  caggacaaca  ctaaatccag  tgcccagcct
1441  gaaaacctga  ttcccatgtg  gtccctctgac  attgtcactg  gggaaaagaa  tgaaccagtg
1501  aagcctctgc  agcccctaat  caaagaacaa  aagccaaagg  accagagtct  tgccctgtcg
1561  cccaagctgg  agtgagtggt  cacaattctg  gctcacagca  acctccgctt  cctgggttca
1621  agtgatttct  cagcctcagc  ctcccgagta  gctgggatta  caggcgtgtg  ccaccacgcc
1681  caggatcaag  ttgctggtga  atgtattata  gaaaaacagg  gcagaatcca  cccagattta
1741  cagccacaca  actctgggtc  tgaaccttcc  ctgtctcgac  agcgacggca  aaagaggaga
1801  gaacagactg  agcacagagg  ggaaaagaga  caggctccga  gagatctctt  tgctttccaa
1861  gagtcgcttc  ctcgattttt  gccttctcat  cccattgttg  ggaaagtgg  tgctacatca
1921  acacaaaaag  aggtgaaaa  ccaacgtaga  gtggtcactg  ggtctgtgag  cagttcaagg
1981  agcagtgaga  tgtcatcatc  aaaggatcga  ccattatcag  ccagagagag  gaggcgacta
2041  aagcagtcac  aggaagaaat  gtccctcttc  ggcccttcag  tgaggaaagc  gtctctgagt
2101  gtagcagggc  caggaaaacc  ccaggaagaa  gaccagccct  tgctgcccgc  acggctctcc
2161  tctgactgca  gcgtcactca  ggaaaggaaa  cagattcatt  gtctgtctga  ggatgagtta
2221  agttcttcta  caagttcaac  tgataagtca  gatggggatt  acggggaaag  gaaaggtcag
2281  acaaatgaaa  ttaatgcctt  ggtacaattg  atgactcaga  ccctgaaact  ggattctaaa
2341  gagagctgtg  aagatgtccc  ggtagcaaac  ccagtgtcag  aattcaaaact  tcatcggaag
2401  tatcgggaca  cactgatact  tcatgggaag  gttgcagaag  aggcagagga  aatccatttt
2461  aaagagctac  cttcagctat  tatgccaggt  tctgaaaaga  tcaggagact  agttgaagtc
2521  ttgagaactg  atgtaattcg  tggcctggga  gttcagcttt  tagagcaggt  gtatgatctt
2581  ttggaggagg  aggatgaatt  tgatagagag  gtacgtttgc  gggagcacat  gggtgaaaag
2641  tatacaactt  acagtgtgaa  agctcgccag  ttgaaatfff  ttgagaaaa  catgaatfff
2701  tgagcatttg  tcctaactgt  ctgccagaat  taaagacct  tttttagagg  tttttggctt
2761  aaaaagcaag  ggcaaacagt  catttggaag  ccactcacca  ctgttttata  tctctttttt
2821  atatctcttt  ggcgtttccc  tacagaaaag  aaattggaca  gaacagaata  atatgaagca
2881  ggatcacaaa  agaaaaaaa  ctttggtctt  catattctct  ttgtgaggac  aaatctgttg
2941  tttgtttgat  tactgtttac  tgagccttaa  tccaccaagt  ttatatttag  aattttatft
3001  ttttaaggta  ctaattaact  taaacacaga  gctataaaat  gctggattga  aaattttata
3061  ttgtaatgta  gagataaaag  cagtaggaga  aacaaatgac  ataatagttc  gtcataatc
3121  ctgctattgt  taataacctt  aaggagtagt  tgataaatta  taaaatttta  aaaaagcaat
3181  tcagttctag  aaatagattt  aaagaatatg  aagttctatc  tagtacttga  gcagctgtat

```

FIG. 16

SUBSTITUTE SHEET (RULE 26)

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```

3241 ttctttttcta cacattgatg gactttttaat attttattct catttaatat aaacctcatc
3301 taggggtatat acaaattaaa actgagacac attggccttg taaatcagta tgtttttaca
3361 taatgggtttt gtttagattta tttttccatc agtgaaaaca tttcttaagc acaaatttca
3421 tttccatttta agcaattttgt aagcaaagtc caggtccatt tagtttttgg atatatattaa
3481 tgttttgtctc ctgaagtttg tcttcatgta ctgtaagata ttagttgtct tttccatgttt
3541 taaatgtatg attatatagc acatatttta ttagttgttt aataagaggt aataccatc
3601 taggaaagaa attttatgaa gttaaataca agtcttgaat agtacatttt cacttctgta
3661 ttcgagggac tctaaaaata aatattgtct cagaaa

```

SEQ ID NO:32

Size: 841

PRT: STK2 (NEK4)

```

1  MPLAAYCYLR VVGKGSYGEV TLVKHRRDGK QYVIKKLNLN NASSRERRAA EQEAQLLSQL
61  KHPNIVTYKE SWEGGDGLLY IVMGFCEGGD LYRKLKEQKG QLLPENQVVE WVFQIAMALQ
121 YLHEKHILHR DLKTQNVFLT RTNIIKVGDL GIARVLENHC DMASTLIGTP YYMSPELFSN
181 KPYNYKSDVW ALGCCVYEMA TLKHAFNAKD MNSLVYRIIE GKLPPMPRDY SPELAELIRT
241 MLSKRPEERP SVRSILRQPY IKRQISFFLE ATKIKTSKNN IKNGDSQSKP FATVVSGEAE
301 SNHEVIHPQP LSSEGSQTYI MGEKCLSQE KPRASGLLKS PASLKAHTCK QDLSNTTELA
361 TISSVNIDIL PAKGRDSVSD GFVQENQPRY LDASNELGGI CSISQVEEEM LQDNTKSSAQ
421 PENLIPMWSS DIVTGEKNEP VKPLQPLIKE QKPKDQSLAL SPKLECSGTI LAHSNLRLLG
481 SSDSPASASR VAGITGVCHH AQDQVAGECI IEKQGRIHPD LQPHNSGSEP SLRQRQRQKR
541 REQTEHRGEK RQVRDLFAF QESPFRFLPS HPIVGKVDVT STQKEAENQR RVVTGSVSSS
601 RSSEMSSSKD RPLSARERRR LKQSQEEMSS SGPSVRKASL SVAGPGKPQE EDQPLPARRL
661 SSDCSVTQER KQIHCLSEDE LSSSTSSTDK SDGDYGEKKG QTNEINALVQ LMTQTLKLDS
721 KESCEDVPVA NPVSEFKLHR KYRDTLILHG KVAEEAEEIH FKELPSAIMP GSEKIRRLVE
781 VLRTDVIRGL GVQLLEQVYD LLEEDEFDR EVRLREHMGE KYTTYSVKAR QLKFFEENMN
841 F

```

FIG. 16
(CONT.)

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SEQ ID NO:33
Size: 1513
DNA NKIAMRE

```

1 atggagatgt atgaaaccct tggaaaagtg ggagaggggaa gttacggaac agtcatgaaa
61 tgtaaacata agaatactgg gcagatagtg gccattaaga tattttatga gagaccagaa
121 caatctgtca acaaaattgc gatgagagaa ataaagtttc taaagcaatt tcatcacgaa
181 aacctgggtca atctgattga agtttttaga cagaaaaaga aaattcattt ggtatttgaa
241 ttatttgacc acacagtatt agatgagtta caacattatt gtcattggact agagagtaag
301 cgacttagaa aatacctctt ccagatccct cgagcaattg actatcttca cagtaataat
361 atcattcatc gagatataaa acctgagaat attttagtat ccagtcagg aattactaag
421 ctctgtgatt ttggttttgc acgaacacta gcagctcctg gggacattta tacggactat
481 gtggccacac gctggtatag agctcccgaa ttagtattaa aagatacttc ttatggaaaa
541 cctgtggata tctgggcttt gggctgtatg atcattgaga tggccactgg aaatccctat
601 cttcctagta gttctgattt ggatttactc cataaaattg ttttgaaagt gggcaatttg
661 tcacctcact tgcagaatat cttttccaag agccccattt ttgctggggt agttcttcct
721 caagttcaac accccaaaaa tgcaagaaaa aaatatccaa agcttaatgg attggttgga
781 gatatagttc atgcttgttt acaaattgat cctgctgaca ggatatcatc tagtgatctt
841 ttgcatcatg agtattttac tagagatgga tttattgaaa aattcatgcc agaactgaaa
901 gctaaattac tgcaggaagc aaaagtcaat tcattaataa agccaaaaga gagttctaaa
961 gaaaatgaac tcaggaaaaga tgaaagaaaa acagtttata ccaatacact gctaagtagt
1021 tcagtttttg gagaggaaat agaaaaagag aaaaagccca aggagatcaa agtcagagtt
1081 attaaagtca aaggaggaag aggagatatt tcagaaccaa aaaagaaaga gtatgaaggt
1141 ggacttggtc aacaggatgc aaatgaaaaa gttcatccta tgtctccaga tacaaaactt
1201 gtaaccattg aaccaccaa ccctatcaat ccagcacta actgtaatgg cttgaaagaa
1261 aatccacatt gcggagggtc tgtaacaatg ccacccatca atctaactaa cagtaatttg
1321 atggctgcaa atctcagttc aaatctcttt caccctcagt tgaggtgagc tgtaacagag
1381 aagaaacctt aataatacaa cattcctgta taatggtatt tcaaagaatc gtgttcatag
1441 tgtctgtatg taaactgaac ttgaagaaaa tatattgaaa ttaaagctgt ataatgggcc
1501 aaaaaaaaaa aaa

```

SEQ ID NO:34
Size: 455
PRT NKIAMRE

```

1 MEMYETLGKV GEGSYGTVMK CKHKNTGQIV AIKIFYERPE QSVNKIAMRE IKFLKQFHHE
61 NLVNLIEVFR QKKKIHLVFE FIDHTVLDEL QHYCHGLESK RLRKYLFIIL RAIDYLSHNN
121 IIHRDIKPEN ILVSQSGITK LCDFGFARTL AAPGDIYTDY VATRWYRAPE LVLKDTSYGK
181 PVDI WALGCM IIEMATGNPY LPSSSDLDDL HKIVLKVGNL SPHLQNI FSK SPIFAGVVL P
241 QVQHPKNARK KYPKLNGLLA DIVHACLQID PADRISSDL LHHEYFTRDG FIEKFMP ELK
301 AKLLQEAKVN SLIKPKESSK ENELRKDERK TVYTNLTLLS SVLGEEIEKE KKPKEIKVRV
361 IKVKGGRGDI SEPKKKEYEG GLGQQDANEN VHPMSPDTKL VTIEPPNPIN PSTNCNGLKE
421 NPHCGGSVTM PPINLTNSNL MAANLSSNLF HPSVR

```

FIG. 17

SUBSTITUTE SHEET (RULE 26)

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SEQ ID NO:35
Size: 3504
DNA HBO1

```

1 gccgctgccc gaatcggaa cgtcggggccg cagccgcccgg caatgcccgcg aaggaagagg
61 aatgcaggca gtagttcaga tggaaaccgaa gattccgatt tttctacaga tctcgagcac
121 acagacagtt cagaaagtga tggcacatcc cgacgatctg ctcgagtacac ccgctcctca
181 gccaggctaa gccagagttc tcaagattcc agtcctgttc gaaatctgca gtcttttggc
241 actgaggagc ctgcttactc taccagaaga gtgaccgta gtcagcagca gcctacocca
301 gtgacaccga aaaaataccc tcttcggcag actcgttcat ctggttcaga aactgagcaa
361 gtggttgatt ttccagatag agaaactaaa aatacagctg atcatgatga gtcaccgcct
421 cgaactccaa ctggaaatgc gccttcttct gagtctgaca tagatatctc cagccccaat
481 gtatctcacg atgagagcat tgccaaggac atgtccctga aggactcagg cagtgatctc
541 tctcatcgcc ccaagcgccg tcgcttccat gaaagctaca acctcaatat gaagtgtcct
601 acaccaggct gtaactctct aggacacct atgagagaca tttctccatc
661 tcaggatgcc cactgtatca taacctctca gctgacgaat gcaagggtgag agcacagagc
721 cgggataagc agatagaaga aaggatgctg tctcacaggc aagatgacaa caacaggcat
781 gcaaccaggc accaggcacc aacggagagg cagcttcgat ataaggaaaa agtggtgaa
841 ctcaagaa gaagaaatctc tggactgagc aaagaacaga aagagaaata tatggaacac
901 agacagacac atgggaacac acgggaacct cttttagaaa acctgacaag cgagtatgac
961 ttggatcttt tccgaagagc acaagcccgg gcttcagagg atttggagaa gtttaaggctg
1021 caaggccaaa tcacagaggg aagcaacatg attaaaacaa ttgcttttgg ccgctatgag
1081 cttgatacct ggtatcattc tccatatcct gaagaatatg cagggtggg acgtctctat
1141 atgtgtgaat tctgtttaaa atatatgaag agccaaacga tactccgccc gcacatggcc
1201 aatgtgtgtg ggaaacaccc acctggtgat gagatatatc gcaaagggtc aatctctgtg
1261 tttgaagtgg atggcaagaa aaacaagatc tactgcaaaa acctgtgcct gttggccaaa
1321 ctttttctgg accacaagac attatattat gatgtggagc ccttctgttt ctatgttatg
1381 acagaggcgg acaacactgg ctgtcacctg attggatatt tttctaagga aaagaattca
1441 ttcctcaact acaacgtctc ctgtatcctt actatgcctc agtacatgag acagggctat
1501 ggcaagatgc ttattgattt cagttatttg ctttccaaag tcgaagaaaa agttggctcc
1561 ccagaacgtc cactctcaga tctggggcct ataagctatc gcagttactg gaaagaagta
1621 cttctccgct acctgcataa ttttcaaggc aaagagattt ctatcaaaga aatcagtcag
1681 gagacggctg tgaatcctgt ggacattgtc agcactctgc aagcccttca gatgtcaaa
1741 tactggaagg gaaaacacct agttttaaag agacaggacc tgattgatga gtggatagcc
1801 aaagaggcca aaagggtcaa ctccaataaa accatggatc ccagctgctt aaaatggacc
1861 cctcccaagg gcacttaaa gacctgtca ttccagcca gcgaacccca gcagtaggaa
1921 tccgtaccct agggatctgt ctgtcatttc tctgttgctc ttgtgattgg caagtacagt
1981 atcctttggg aaggccatcc cctcaggac tgcctggct ccgaccttg tgtacactgc
2041 agcgtctggt tctgaggaac tgtttgttcg gcctcagtg ggttgcttgg atgggactgt
2101 tattagactt gagtgcaggt ctctcagcac tgacccaagg agttctgtta tggtaactgt
2161 cctgtccagt cactggttct ctctcatgt cctctcgccc catgagggtt tgttgtgtct
2221 tctaagcgtg gtactagtgc ttgccacctg gtcaccagac ctccaaatat ggctgccacc
2281 accaggacct ttccagttac tccttatatg tgtgttctat ggaggggcag ggaaaagggtg
2341 gcacttgtga gtgtgtgtgg attggcaggg ggtccattca ctttgggttc catcttgctt
2401 taaatttctt catttttgatt aagagacct tttttgatct gtattgggct aaccagagcc
2461 aaatactttt gaagagtttc ccagggacta gtcattggtaa tagcatataa ttgatctgaa
2521 tgagatggag agaagaatga aggggtggtg gttctgggtt tgatttgagt tcacctgttg
2581 gcagtgggca gtgggcagtg tcttggtgaa agggaaacgga tactactttt tgctcaccg
2641 taaagtactc actagtaaat atttccctct ctctttactc ccacttttta cgtttgcagg
2701 tgccaaagta atgtccactt ttccctttca tgctgcatat taactgggta attatactgc
2761 agaaaccttt tcacctccac tagtctgata cagtacatct gtacttccat ataccttgca
2821 ctgattttgt ctgagtgcct tgggagaagt agaaaatgat tgaaagtgac tccgtatct
2881 cagcccatga ctcagcaagg cagaaatggc acccctgcca aagtttgctt ctcttttcaa
2941 cagtgcctca cctccctctt aggatataag tgcttctgcc ctccacgaa ctctcctcc
3001 atttcccttt tgggatttgt caccatcctt ctattctctg gtcttctatt tttgggtgtg
3061 ttcaagtga ggaagagatg ttccctctaa tttctctcta gccattata acctgctatc
3121 ttggggcaac ttttgatgta tgacatgtca ccttcccaa cttggtctcc tccaacatgc
3181 tgtcttcatg tggagccctc accacaatcc ctgactccgg tcatttgtgc ctttctcttg

```

FIG. 18

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```
3241 tcattctctgt acactactta tattcactgt gggttggggg agctaatttt aagcatgttc
3301 agtggcagct cccctccagt ttcagtgtca ctgttaaaat ttatcaaaaa gcaacttcac
3361 taggggtttt cttaagggat aaaggccttt tacagaagct aaacccttcc ccacatgtgg
3421 tagaatgtgc tcttctatat ctactcctca ataaagcatg ttctctgctc aaaaaaaaaa
3481 aaaaaaaaaa aaaaaaaaaa aaaa
```

SEQ ID NO:36
Size: 611
PRT HBO1

```
1 MPRRKRNAGS SSDGTEDSDF STDLEHTDSS ESDGTSRRSA RVTRSSARLS QSSQDSSPVR
61 NLQSFgteep AYSTRRVTRS QQQTPVTPK KYPLRQTRSS GSETEQVVDf SDRETKNTAD
121 HDESPPrTPT GNAPSSesDI DISSPNVSHD ESIakDMSLk DSGSDLShRP KRRRFHESYN
181 FNMKCPTPGC NSLGHLTGKH ERHFSISGCP LYHNLSADEc KVRAQSRDKQ IEERMLShRQ
241 DDNNRHATRH QAPTERQLRY KEKVAELRKK RNSGLSKEQK EKymeHRQTY GNTREPLEN
301 LTSEYDLDLF RRAQARASEd LEKLRLQGQI TEGSNMIKTI AFGRYELDTW YHSPYPEEYA
361 RLGRLYMCEf CLKYMKsQTI LRRHMAKCVW KHPPGDEIYR KGSISVFEVD GKKNKIYCQN
421 LCLLAKLFLD HKTLYYDVEP FLfYVMTEAD NTGCHLIGYf SKEKNSFLNY NVSCILTMPQ
481 YMRQGYGKML IDfSYLLSKV EEKVGSPERP LSDLGLISYR SYWKEVLLRY LHNfQGKEIS
541 IKEISQETAV NPVDIVSTLQ ALQMLKYWKG KHLVLKRQDL IDEWIAKEAK RSNSNKTMdP
601 SCLKWTPPKG T
```

**FIG. 18
(CONT.)**

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Gene Name	Accession (nt/aa)	Screen	Activity
PKC-zeta	NM_002744/ AAA36488	ATM ip	S/T kinase
PLC-beta I	NM_01519/ NP_056007	RbAp48 ip	Phospholipase
PTK2(FAK)	L05186/AAA35819	14-3-3 YTH	Y kinase
PTK2b(FAK2)	L49207/ Q14289	XIAP YTH	Y kinase
CK2	NM_001895/ NP_001886	DNAPK YTH	S/T kinase
cMET	J02958/AAA59591	RbAp48 ip	Y kinase
FEN1	NM_004111/ NP_004102	PCNA YTH	Endonuclease
REV1	AF206019/ AAF18986	Myt1 YTH	dCMP transferase
APE1:	X66133/S34422	p16 YTH	Endonuclease
CDK3:	NM_001258/ NP_001249	CKS2, HSPC YTH	S/T kinase
PIM1	M16750/AAA60089	p21 ip	S/T kinase
CDC7L1	NM_003503/ NP_003494	Apoptin, GADD34 YTH + bioinf	S/T kinase
CDK7	NM_001799/ NP_001790	CIP1 YTH+bioinf	S/T kinase
CNK	NM_004073/ NP_004064	DNAPK F7 YTH	S/T kinase
PRL-3	NM_007079/ NP_009010	Myt1 YTH	Y phosphatase
STK2	XM_003216/ XP_003216	p73 YTH	S/T kinase
NKIAMRE	AF130372/ AAF36509	RbAp48 ip	S/T kinase
HBO1	NM_007067/ NP_008998	p66H YTH	Histon acetylase

FIG. 19

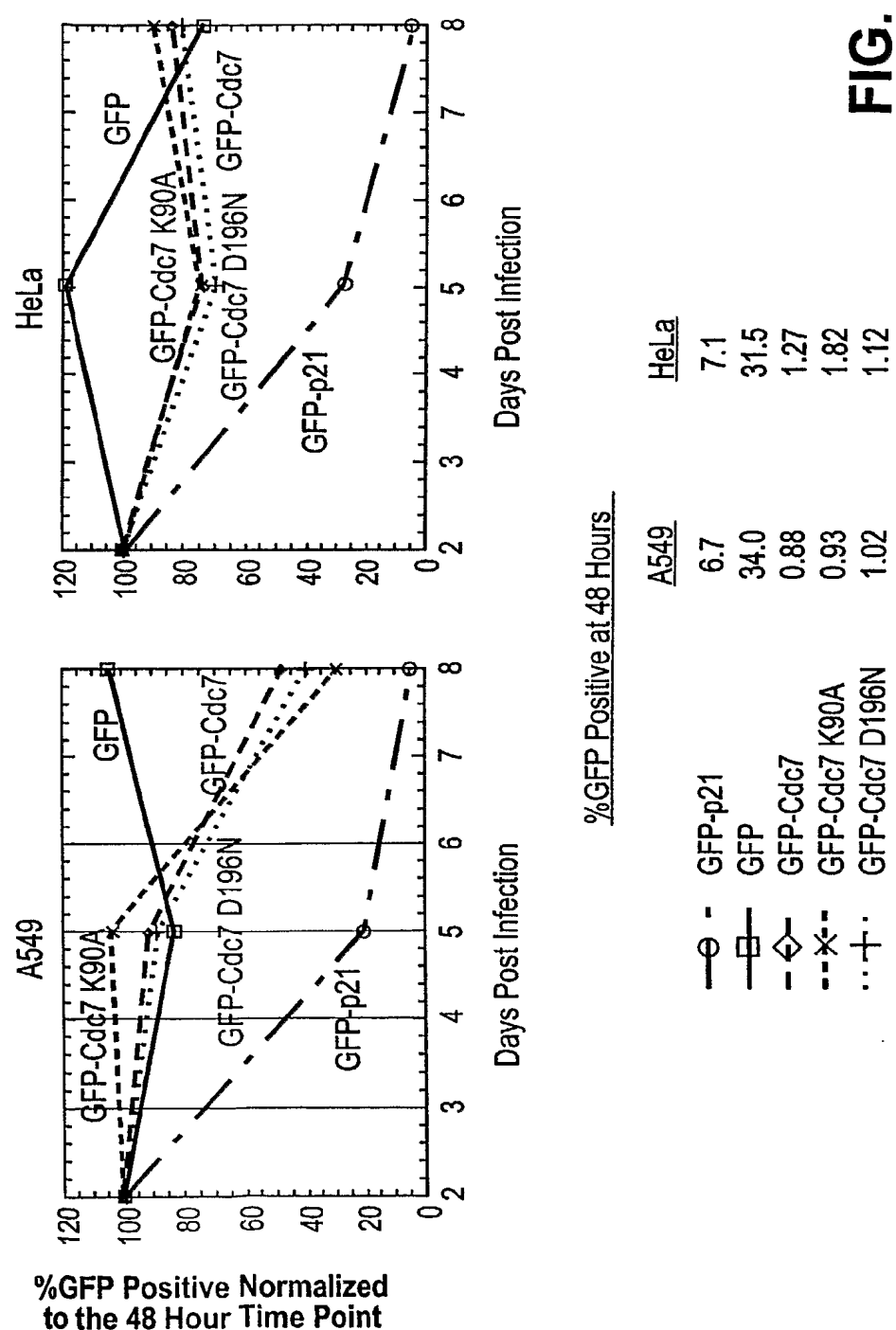


FIG. 20

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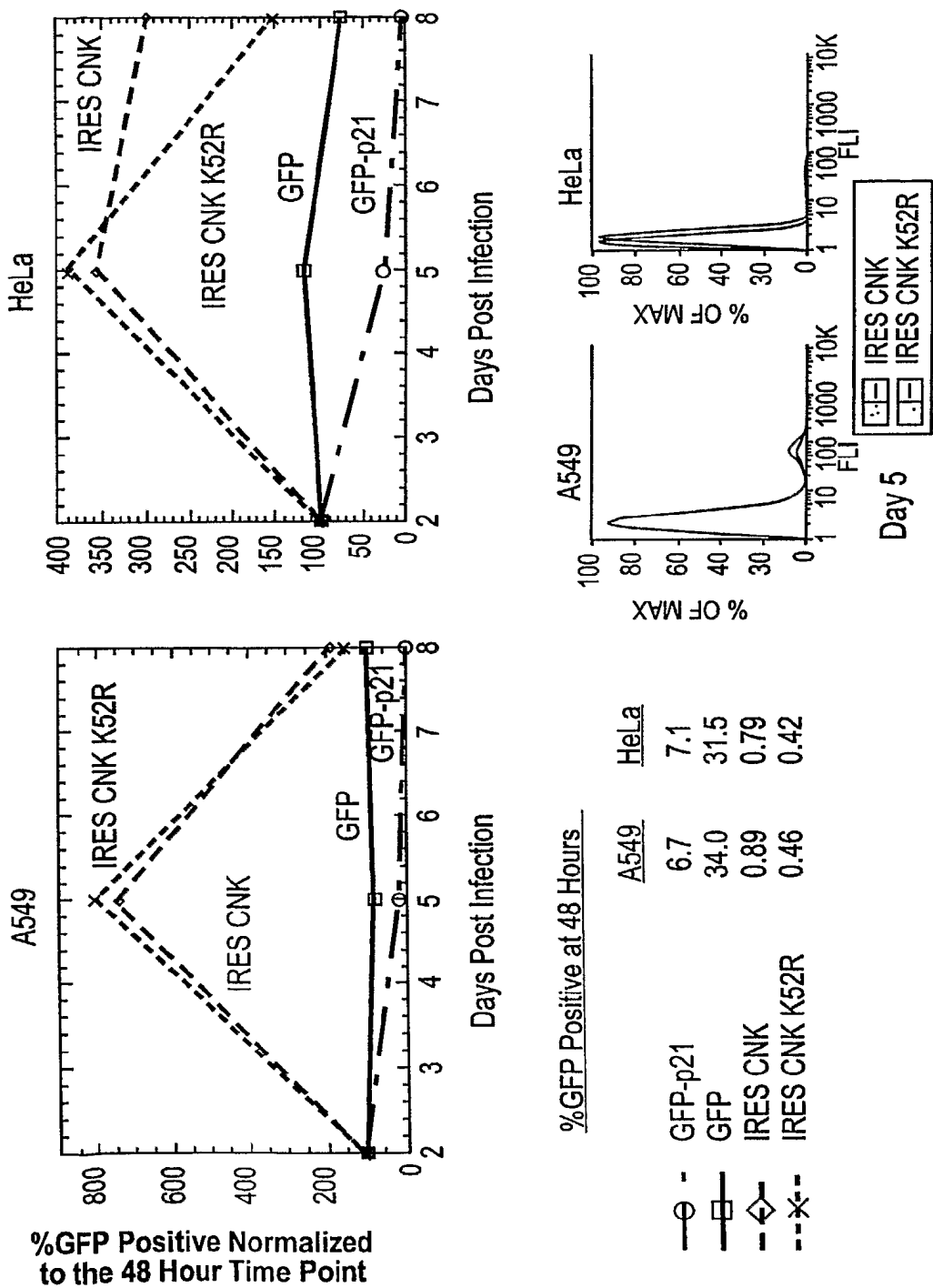
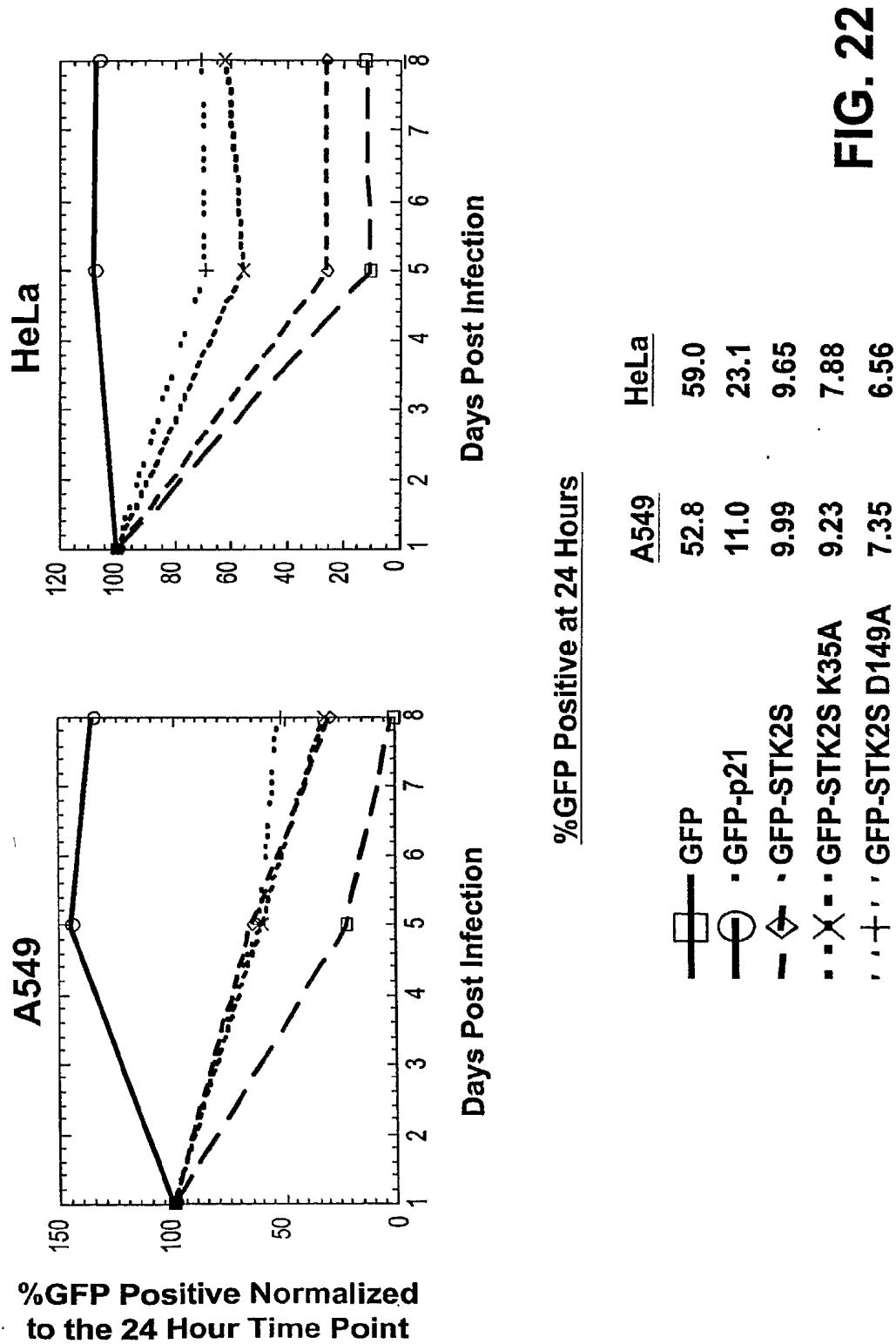


FIG. 21



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Point mutant : K90A, D196N - K90A corresponds to a mutation in the catalytic residue in the kinase domain
 D196N is a mutation in the activation loop of the kinase domain. (Mol Gen Genet. 1997 May 20;254(5):562-70.PMID: 9197416)

CDC7L1	MEASLGIQMDPEPMAFSPQDRDFAEGSLKKNEQNFKLAVGKKDIEKLYEAVPQLSNVFKI
CDC7Sc	-----MTSKTKNIDDIIPPEIKEEMIQLYHDLPGLIENEYKL
	: *::: : .::: : *::: : *::: : *::: : *
CDC7L1	EDKIGEGTFSSVYLAT-----AQLQVGPEEKIALKHLIPTSHPIRIAAELQCLT
CDC7Sc	IDKIGEGTFSSVYKAKDITGKITKKFASHFWNYGSNYVALKKIYVTSSPQRIYNEINLLY
	***** * . : : : : : * * * * *
CDC7L1	VAGGQDNVMGVKCYCFRKNNDHVVIAMPYLEHESFLDILNSLSFQEVREYMLNLFKALKRIH
CDC7Sc	IMTGSSRVAPLCDAKVRDQVIAPLPYPHEEFRTFYRDLPIKGIKKYIWELLRALKFVH
	: *... * : . * *::: : * * * * : ... * : : : : * * * * *
CDC7L1	QFGIVHRDVKPSNFLVNRRLKKYALVDFGLAQGTHDTKIELLKVFQSEAQQERCSONKSH
CDC7Sc	SKGIIHRDIKPTNLFNLELGRGVLVDFGLAEAAQMDYKSMISSQND-----
	. **::: **::: **::: * : . *****:: * * : . :
CDC7L1	IITGNKIPLSGPVPKELDQQSTTKASVKRPTYNAQIQIKQKDGKESVGLSVQRSVPGE
CDC7Sc	-----YDN-----YANTNHDGGYSMRNHEQFCPC
	: . : . : * . : . : . : .
CDC7L1	RNFNIHSSISHESPAVKLMKQSKTVDVLSRKLATKKKAISTKVMNSAVMRKTASSCPASL
CDC7Sc	IMRNQYSPNSHNQTPPMVTIQNGKVHLN-----
	* : * . **::: . : * . . * *
CDC7L1	TCDCYATDKVCSICLSRRQQVAPRAGTPGFRAPEVLTKCPNQTTAIDMWSAGVIFLSLLS
CDC7Sc	NVNGVDLTGYPKNETRRIKRANRAGTRGFRAPEVLKCGAQTIDKIDWSVGILLSLLG
	. : * . : * * * * * ***** ** *::: *::: *::: *::: *::: *

FIG. 23

Point mutant : K52R and D146A - the catalytic residue in the kinase domain.
(J. Biol. Chem., Vol. 276, Issue 46, 43305-43312, November 16, 2001. PMID: 11551930)

pkinase: domain 1 of 1, from 23 to 275: score 309.5, E = 2.5e-90

```

      *->yelleklGeGsfGkVykakhkdkgtkiVAVKilkkakesikek...r
      y +++ lG+G+f+++y+a++ +tg +AVK+++ + + k+++++
query  23  YLKGRLLGKGGFARCYEATDT-ETGSAYAVKVIP-QSRVAKPHqreK 67

      flrEiqilkrLsHpNIvrligvfedtdhhlylvmEymegGdLfdylrrng
      +l+Ei++++ L+H +Ivr+ + fe + d++y+ +E++ +L+++++++
query  68  ILNEIELHRDLQHRHIVRFSHHFE-DADNIYIFLELCRSKSLAHIWKARH 116

      gplsekeakkialQilrGleYLHsngivHRDLKpenILldendgtvKiaD
      + l e+e++ + +Qil+Gl+YLH +gi+HRDLK N+++++en + +K++D
query  117 TLL-EPEVRYLRLQILSGLKYLHQRGILHRDLKLGNGFFITEN-MELKVG 164

      FGLArllle.sssklttfvGtpwYmmAPEvilegrgysskvDvWSlGvily
      FGLA+ le++ ++++t++Gtp+Y+ APEv l+++g+++++DvWSlG+++y
query  165 FGLAARLEpPEQRKKKTICGTPNYV-APEV-LLRQGHGPEADVWSlGCVMY 212

      El1tggplfpgadlpaftggdevdqliifvlklPfsdelpktridpleel
      l1+g +Pf+ + l+e
query  213 TLLCG-----SPFFE-----TADLKET 229

      friikrpglrlplpsncSeelkdLlkkclnkDPskRpGsatakeilnhpwf
      +r ik+ ++ lp ++S ++++Ll +L+ P +Rp ++ +il h +f
query  230 YRCIKQ--VHYTLPASLSLPARQLLAAILRASPRDRP---SIDQILRHDF 275

```

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FIG. 24

Point mutants : K35A and D149A - the catalytic residue in the kinase domain.

```

pkina: domain 1 of 1, from 6 to 261: score 288.9, E = 4.2e-84
      *->yelleklGeGsfGkVykakhkdkgtgiVAvKilkkakesikek...r
      y  l++G+Gs+G+V ++kh+ +gk++++K+l+ ++ ++++++
query   6  YCYLRVVGKGSYGEVTLVKHR-RDGKQYVIKKLN--LRNASSRerrA 49
      ●
      flrEiqilkrLsHpNivrligvfedtdhlylvmEymegGdLfdylrrng
      +  E+q+l +L+HpNiv+++++e d+ ly+vm ++egGdL++ l++++
query  50  AEQEAQLLSQLKHPNIVTYKESWEGDGLLYIVMGFCEGGDLYRKLEQK 99
      ●
      .gpIsekeakkialQilrGleYlHsngivHRDLKpeNIlldendgtvKia
      +  l+e++++ ++ Qi+ +l+YLH+++i+HRDLK++N++l++ + +K++
query  100  GQLLPENQVVEWFVQIAMALQYLHEKHILHRDLKTQNVFLTRT-NIIKVG 148
      ●
      DFGlArllle.sssklttfvGtpwYmmAPEvilegrgysskvDvWSlGvIL
      D  G+Ar+le++ +++t+ Gtp+Ym +PE+ ++++y k+DvW+lG+ +
query  149  DiGIARVLEnHCDMASTLIGTPYm-SPEL-FSNKPYNYKSDVWALGCCV 196
      ●
      yElltgglfpgadlpafgtggdevdqliifvklPfsdelpktridplee
      yE+++          k  f          +d+ +
query  197  YEMATL-----KHAfNA-----KDMNSL 214
      ●
      lfriikrpglrlplpsncseelkdLlkkcLnkDpskRpGsatakeilnhpwf<-*
      ++rii++ ++p p+ S+el +L++ +L k P++Rp + + il p++
query  215  VYRIIEG--KLPPMPRDYSPELAELIRTMlSKRPERP---SVRSILRQPYI 261

```

FIG. 25

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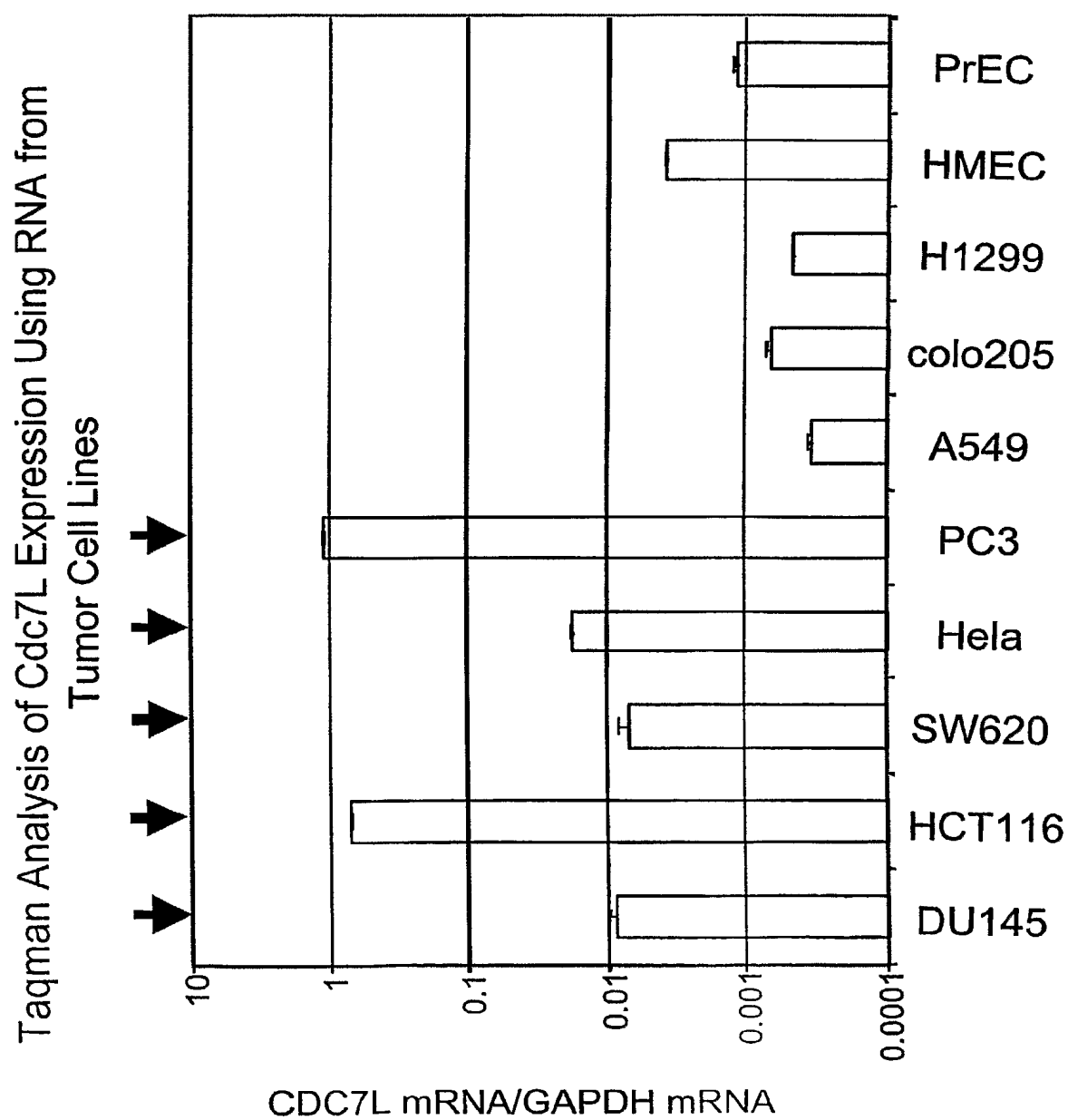
Dominant Negative Mutants for Cdc7L1

Point mutant : K90A, D196N - K90A corresponds to a mutation in the catalytic residue in the kinase domain D196N is a mutation in the activation loop of the kinase domain. (Mol Gen Genet. 1997 May 20;254(5):562-70.PMID: 9197416)

CDC7L1	MEASLGIQMDEPMAFSPQDRFQAEGLSKKNEQNFKLAGVKKDIETKLYEAVPQLSNVFKI
CDC7Sc	-----MTSKTKNIDIPPEIKEEMIQLYHDLPGIENEYKLI
	: *::: : . *::: : *::: : *::: : *::: : *
CDC7L1	EDKIGEGTFSSVYLAT-----AQLQVGPEEKIALKHLIPTSHPIRAAELQCLT
CDC7Sc	IDKIGEGTFSSVYKAKDITGKITKKFASHFNWYGSNYVALKKIYVTSSPQRIYNELNLLY
	***** * . ::: : *::: : *::: : *::: : *::: : *
CDC7L1	VAGGQDNVMGVKCYCFRKNQDVHVIAMPYLEHESEFLDILNSLSFQEVREYMLNLFKALKRIH
CDC7Sc	IMTGSSRVAPLCAKRVRDQVIAPLPYPHEEFRTFYRDLPIKGIKKYIWELLRAKLFVH
	: *... * : . *::: : *::: : *::: : *::: : *::: : *::: : *
CDC7L1	QFGIVHRDVKPSNFLYNRRLLKKYALVDFGLAQGTHDTKIELLKFFVQSEAAQQERCSQNKSH
CDC7Sc	SKGIIHRDIKPTNLFNLELGRGVLD FGLAEAQMDYKSMISSQND-----
	. **::: **::: **::: * . * : . *****:.. * * : . : *
CDC7L1	IITGNKIPLSGVPKELDQOSTTKASVKRPTYNAQIQIKQKDGKEGSGVGLSVQRVSFGE
CDC7Sc	-----YDN-----YANTNHDGGYSMRNHEQFCPC
	: . : . : *::: : *::: : *::: : *
CDC7L1	RNFNIHSSISHESPAVKLMQSKTVDVLSRKLATKKKAISTKVMNSAVMRKTASSCPASL
CDC7Sc	IMRNQYSPNSHNQTPPMVTIQNGKVVHLN-----
	* : * . *::: : *::: : * . * . * *
CDC7L1	TCDCYATDKVCSICLSRRQQVAPRAGTPGFRAPVLTCPNQTTAIDMWSAGVIFLSLLS
CDC7Sc	NVNGVDLTKGYPKNETRRIKRNAGTRGFRAPVLMKCGAQSTKIDIWVGVILLISLLG
	. : * . : *::: : *::: : *::: : *::: : *::: : *::: : *

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FIG. 27



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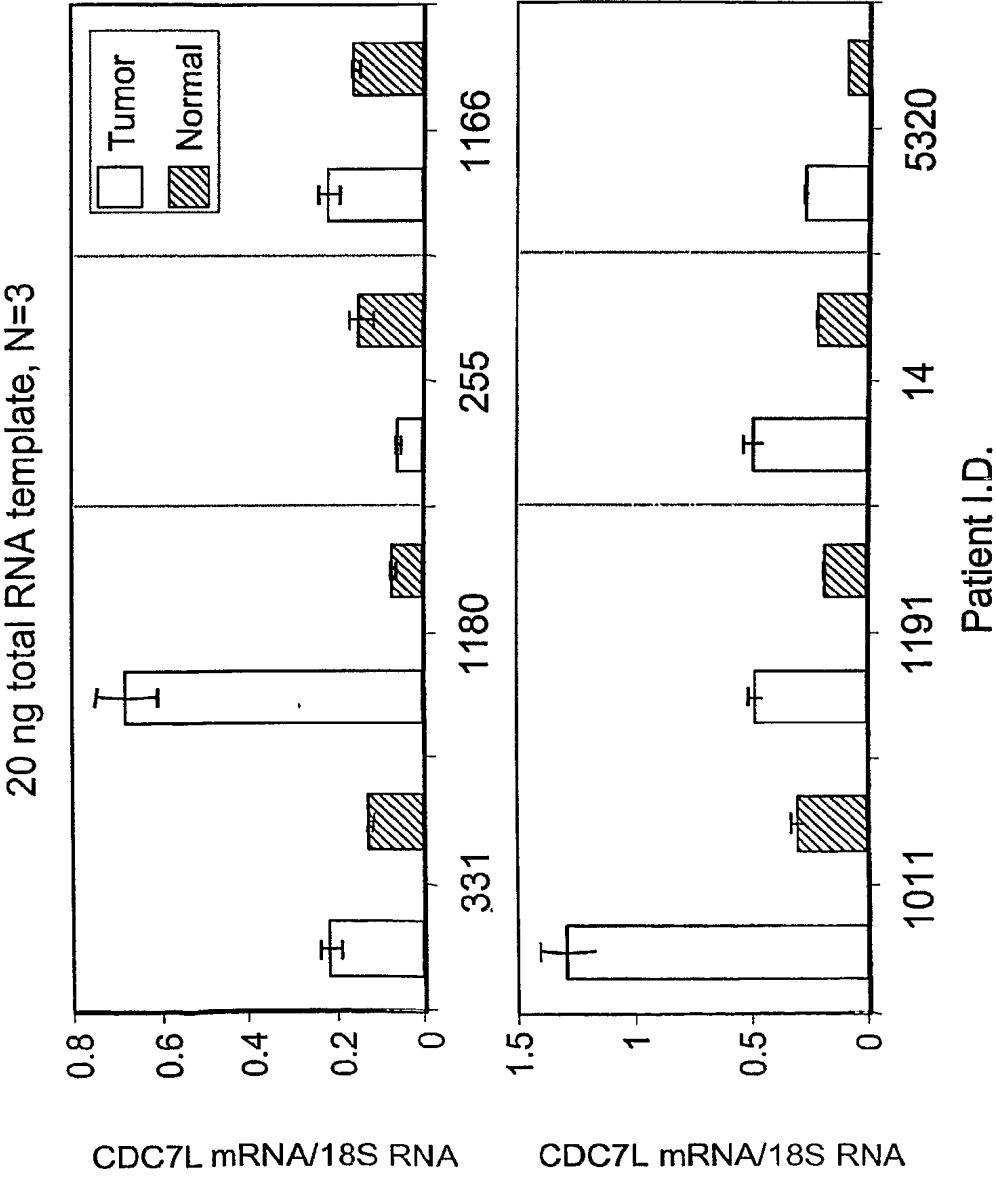


FIG. 28

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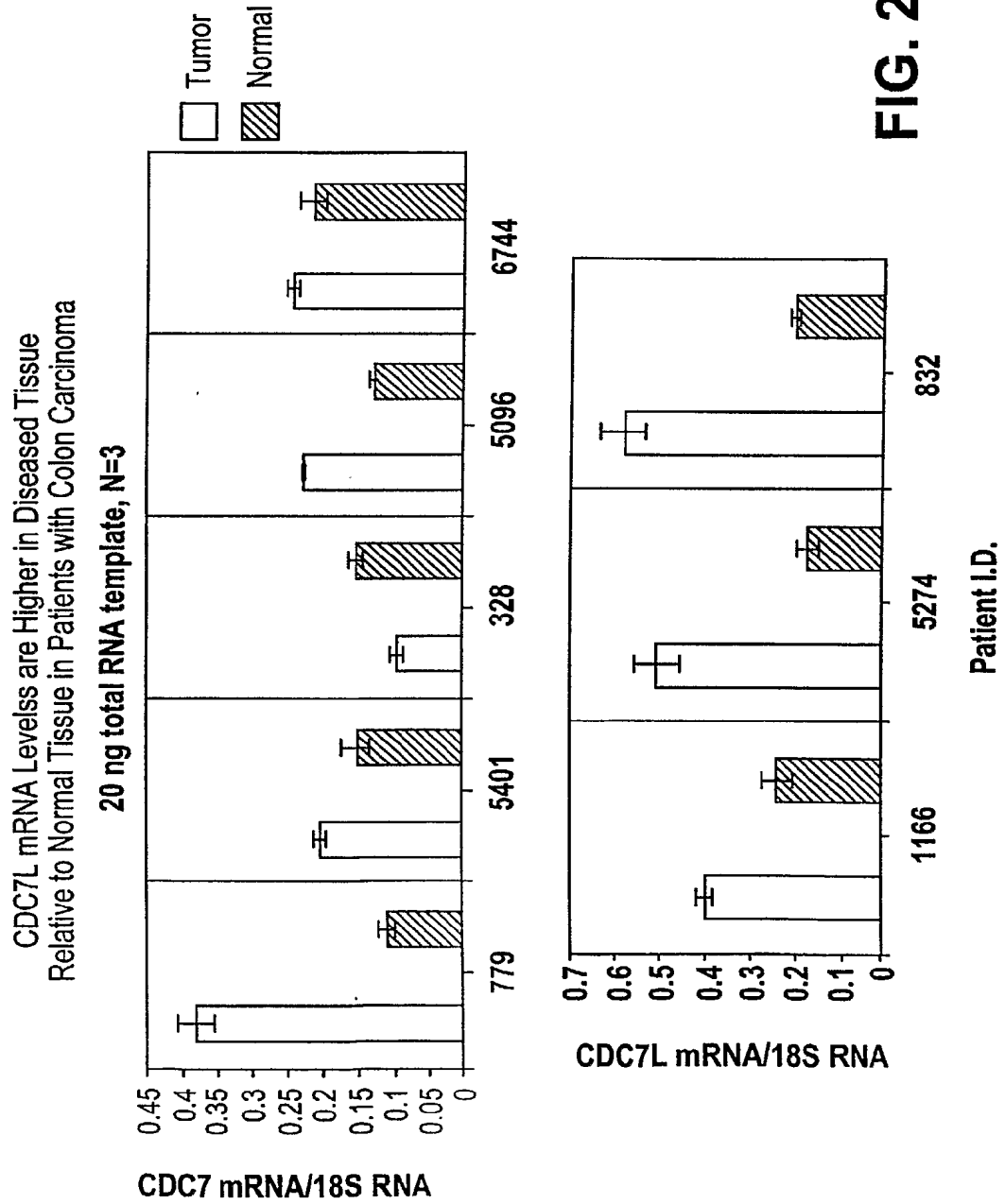


FIG. 29

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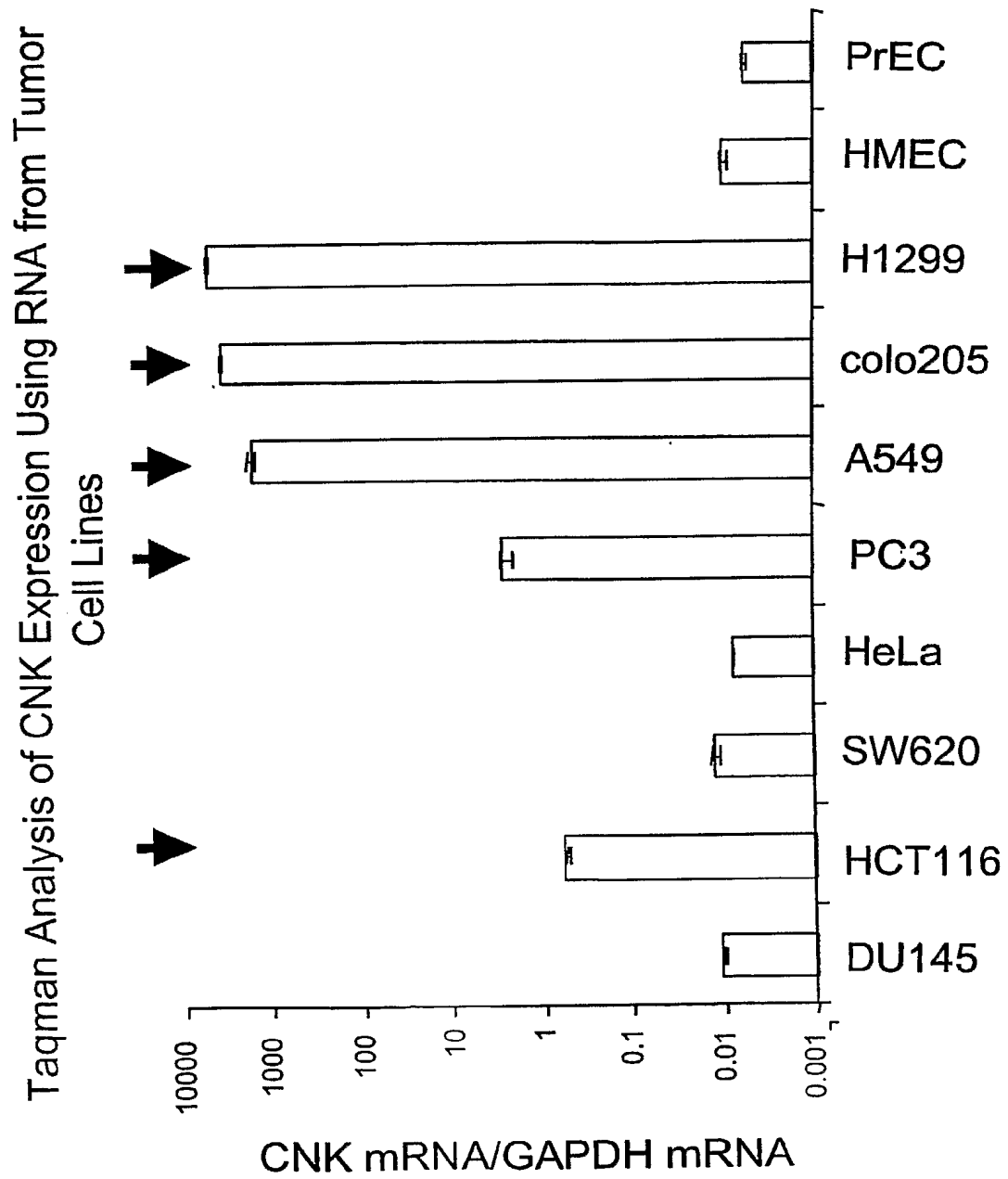
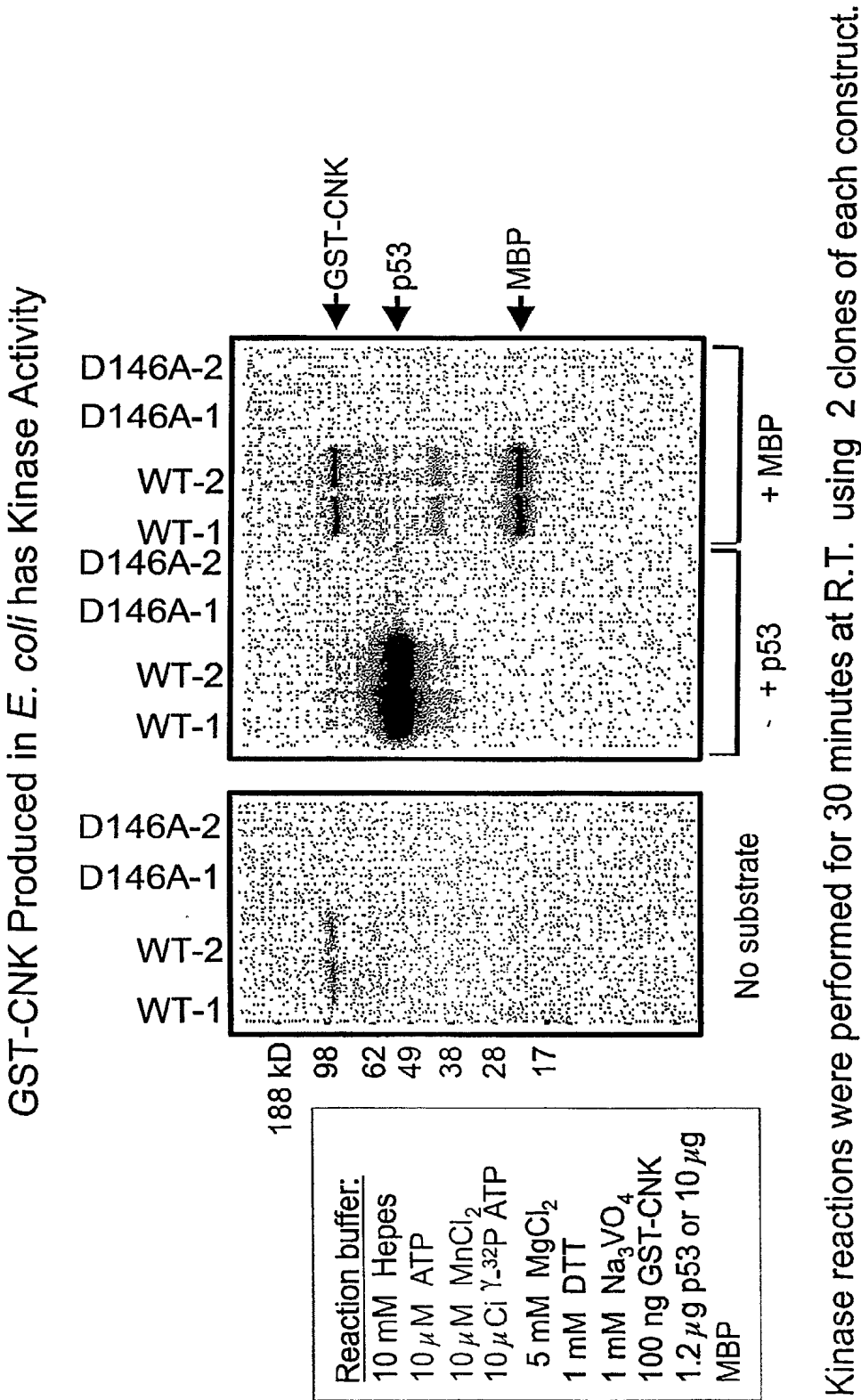


FIG. 30

N=3, 20 ng total RNA template sample



RT-PCR of mRNAs from Different Tissues and Cell Lines Suggests that the Larger STK2 Isoforms Predominates in *H. sapiens*

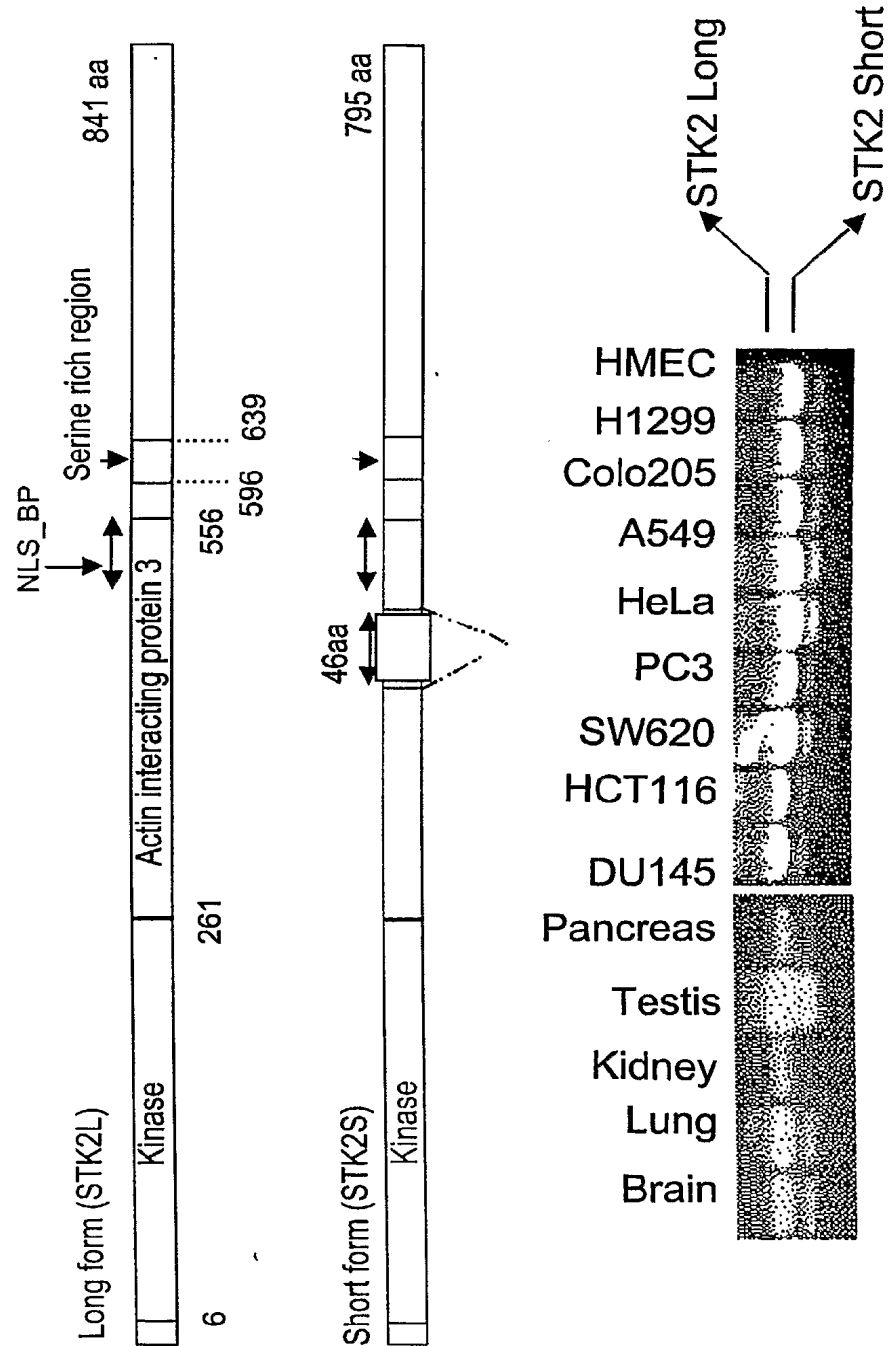


FIG. 32

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Taqman Analysis of STK2 Expression using RNA from Tumor
Cell Lines

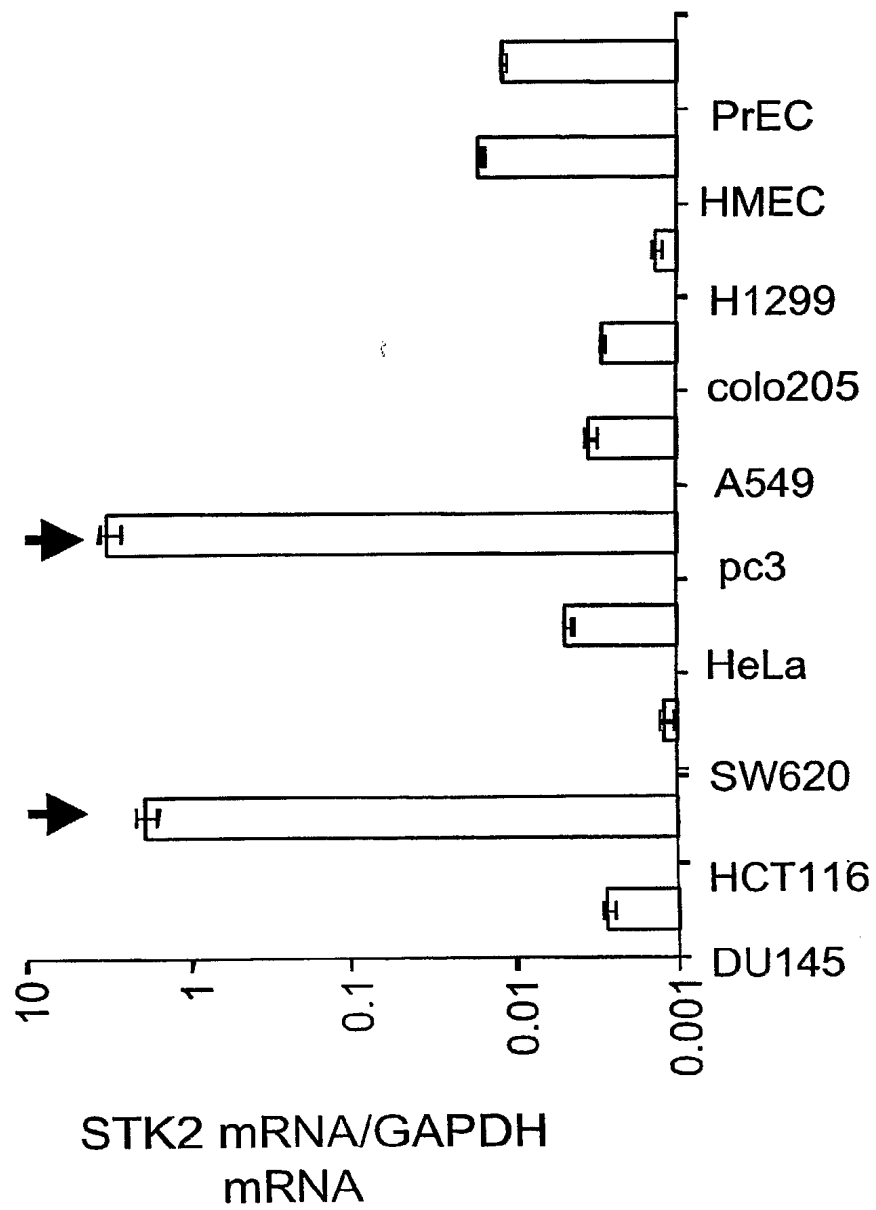
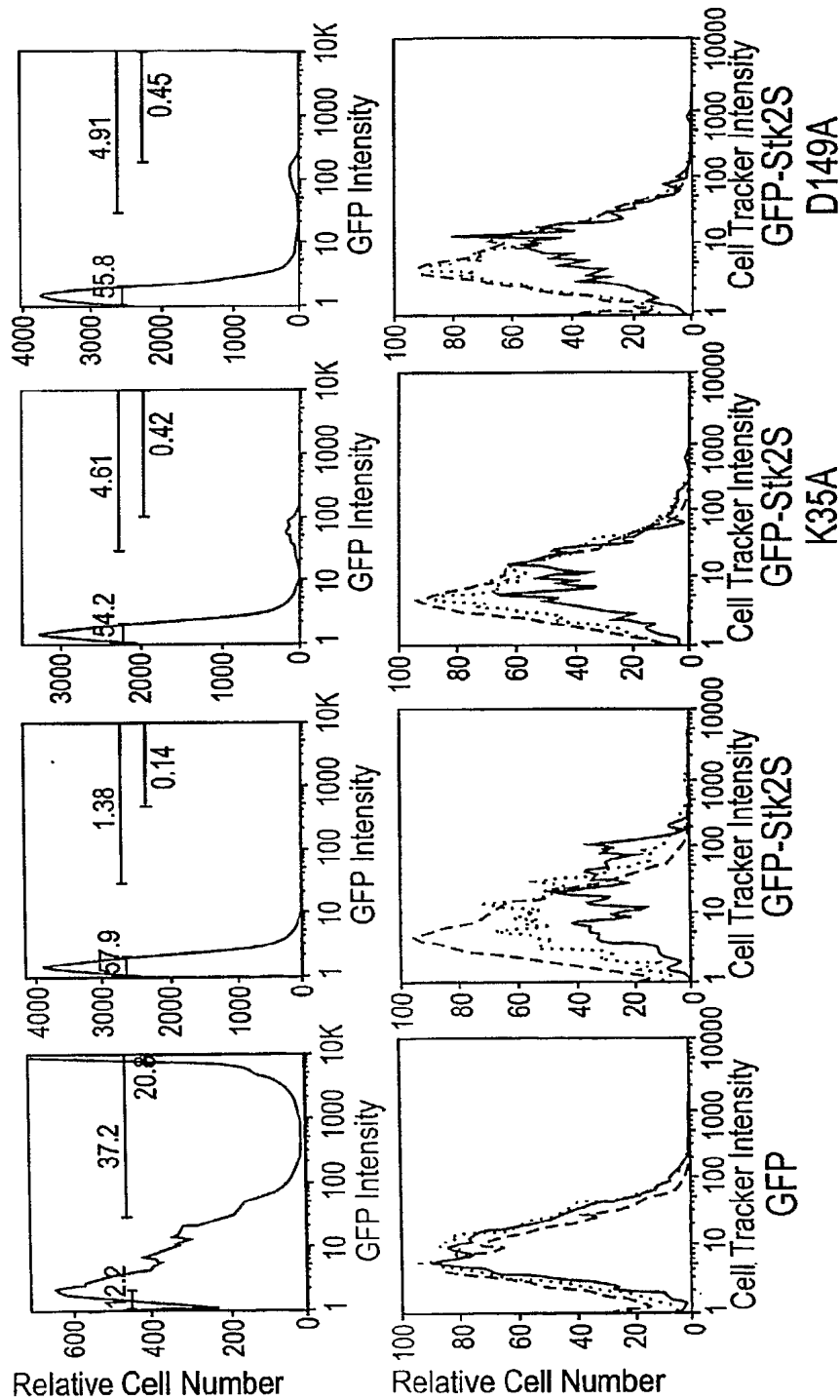


FIG. 33

N=3, 20 ng total RNA template sample

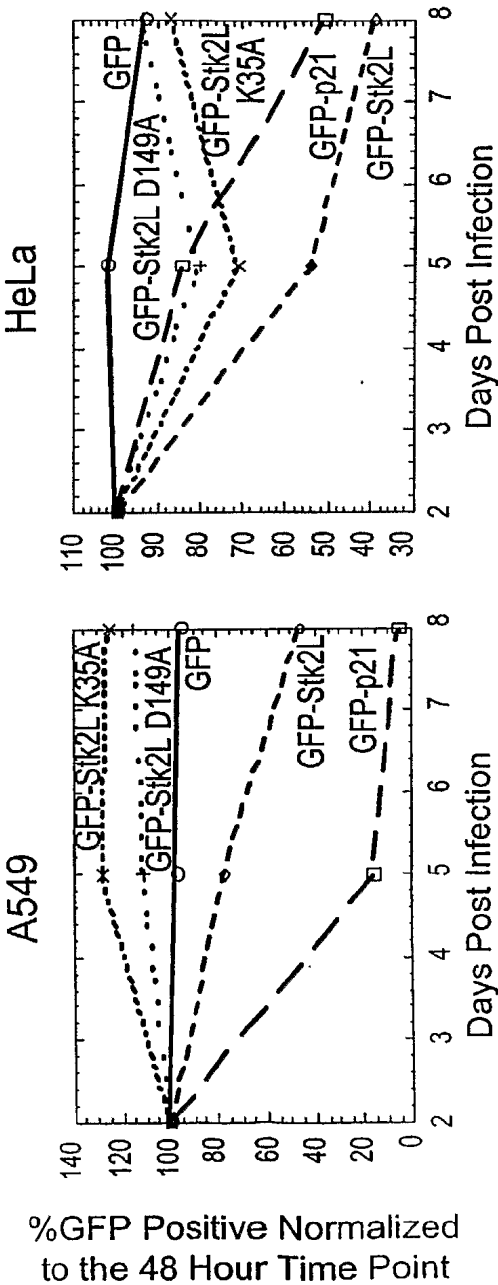
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GFP-STK2 Short is Antiproliferative when Measured Using the Cell Tracker Assay



Cell Tracker Assay Day 8 A549 GFP Pos. — — GFP Neg. — — GFP Hi **FIG. 34**

Expression of GFP-Stk2 Long is Antiproliferative in A549 and HeLa Cells



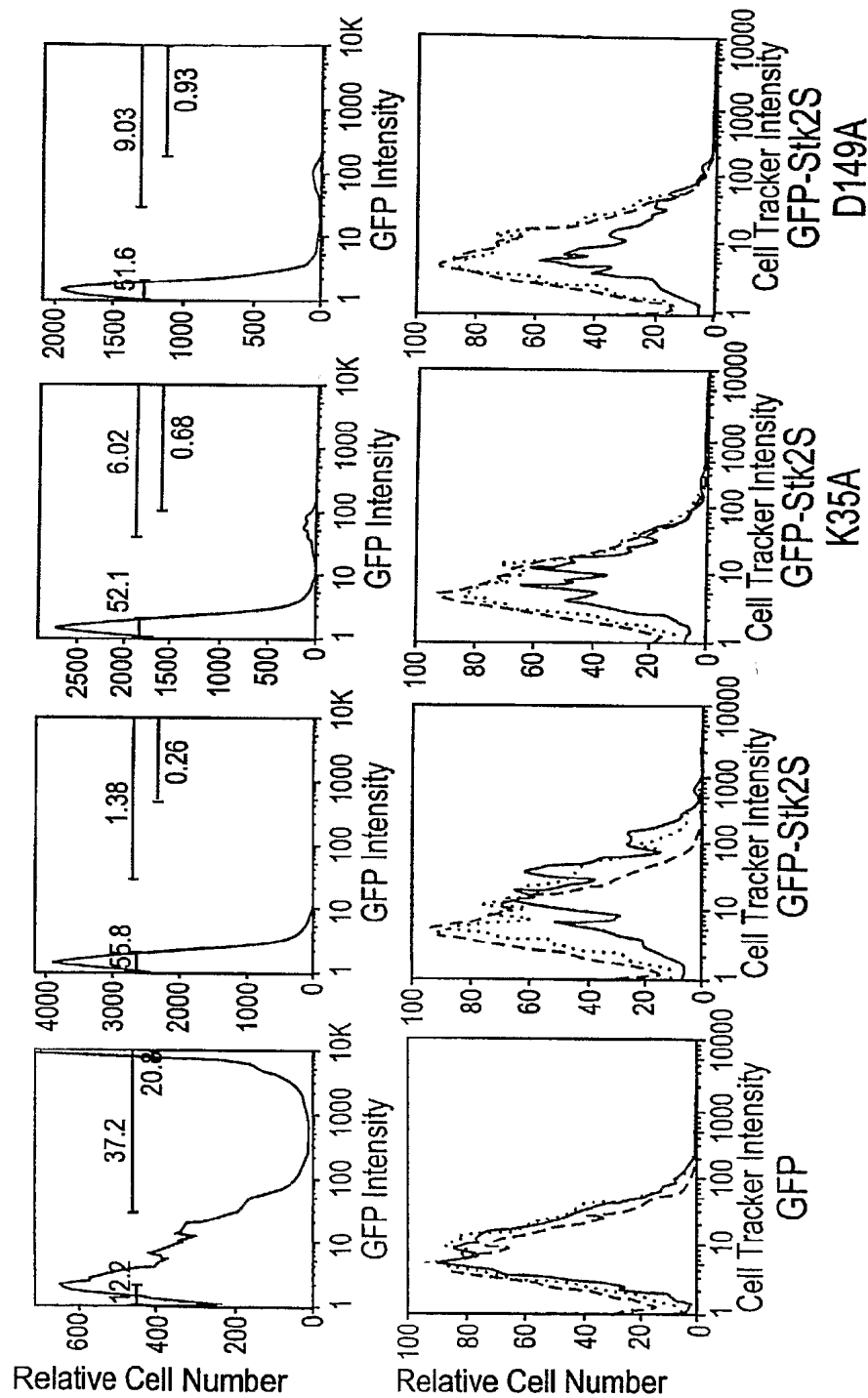
%GFP Positive at 48 Hours

	A549	HeLa
GFP	39.0	48.6
GFP-p21	8.5	4.2
GFP-Stk2L	5.5	6.1
GFP-Stk2L K35A	4.9	7.3
GFP-Stk2L D149A	7.9	3.4

FIG. 35

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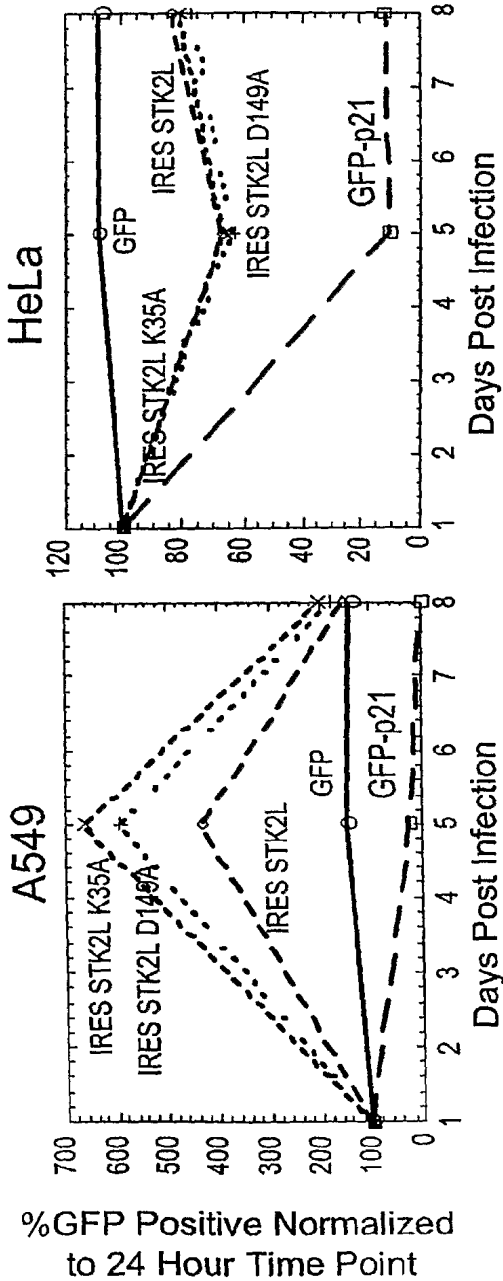
GFP-STK2 Long is Antiproliferative when Measured Using the Cell Tracker Assay



Cell Tracker Assay Day 8 A549 GFP Pos. — — GFP Neg. — — GFP Hi **FIG. 36**

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Expression of STK2L WT and Mutants Using IRES Vectors is Antiproliferative in A549 Cells



%GFP Positive at 24 Hours

	A549	HeLa
GFP	52.8	59.0
GFP-p21	11.0	23.1
IRES STK2L	0.80	0.64
IRES STK2L K35A	1.79	2.3
IRES STK2L D149A	1.93	4.4

FIG. 37

IRES Hbo1 E508Q is Antiproliferative in A549 Cells

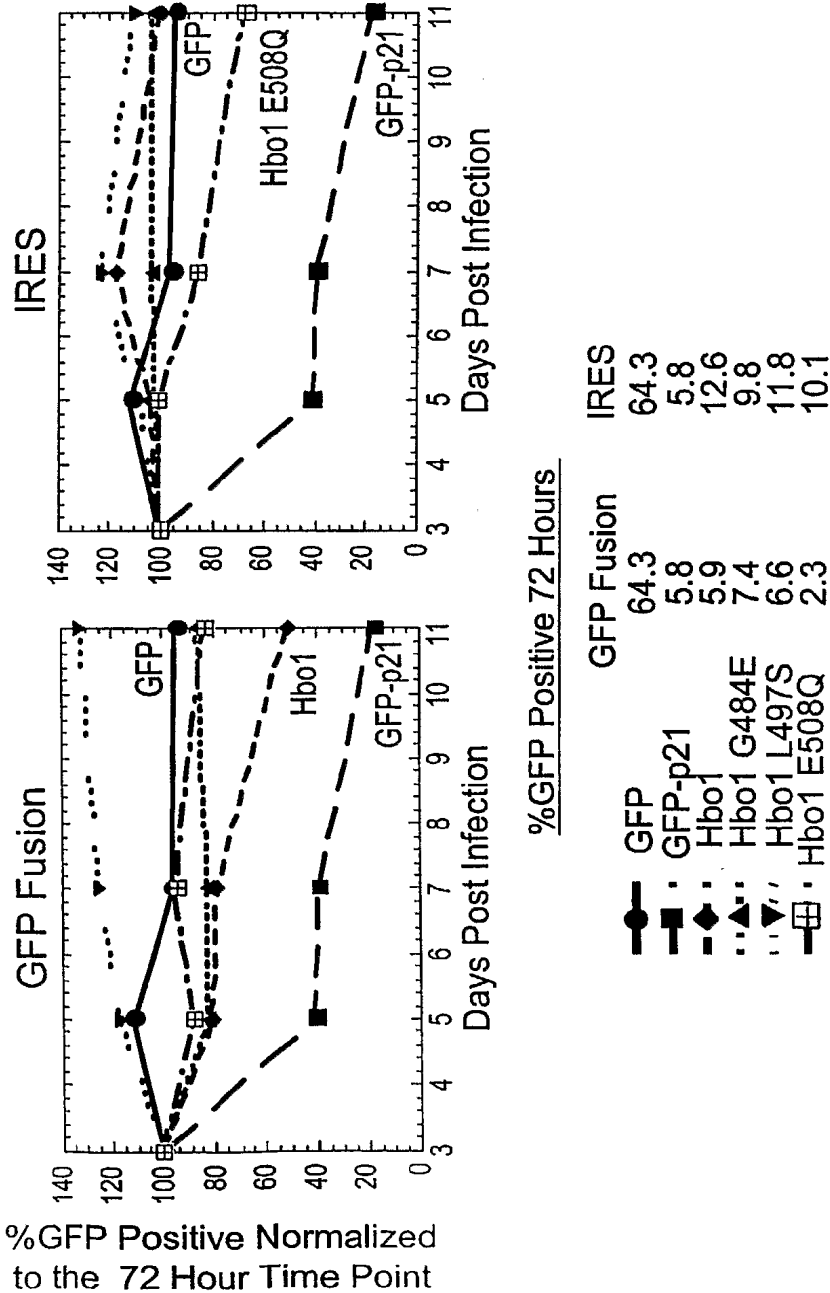
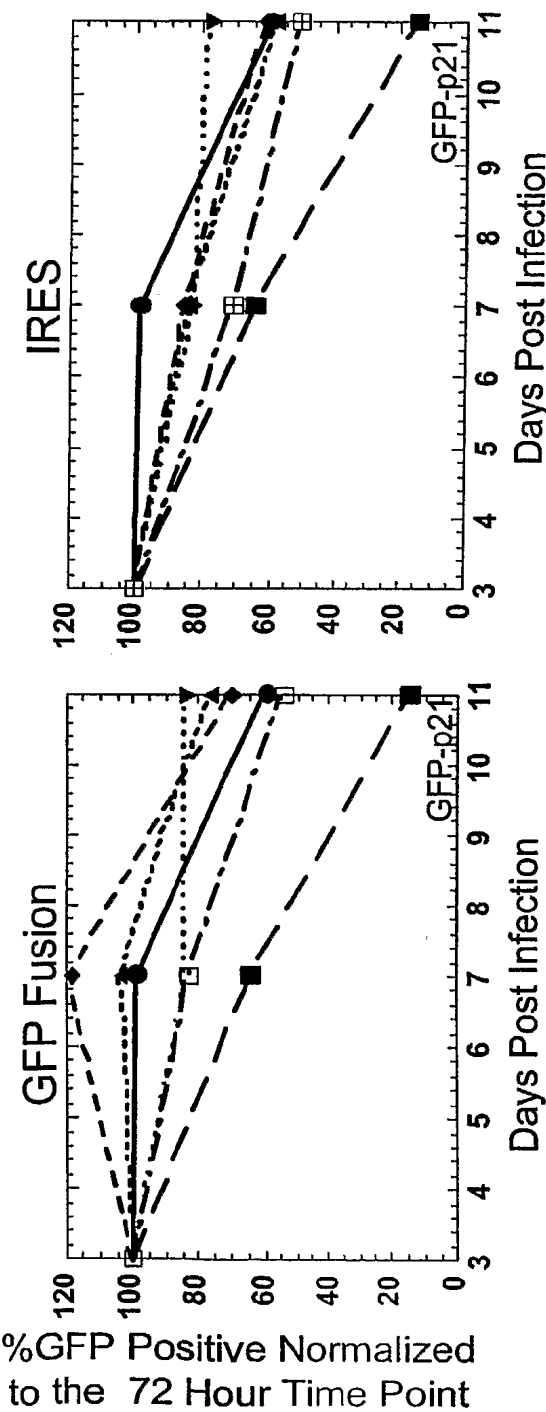


FIG. 38

GFP-Hbo1 has a dominant negative effect which is not observed with the IRES construct

No Significant Differences Are Observed Between Hbo1 WT and Mutant Proteins in H1299 Cells



%GFP Positive 72 Hours		
GFP Fusion	IRES	
GFP	29.2	29.2
GFP-p21	1.3	1.3
Hbo1	3.3	2.5
Hbo1 G484E	6.2	3.4
Hbo1 L497S	3.8	4.5
Hbo1 E508Q	3.2	2.2

FIG. 39

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Hbo1 E508Q is Antiproliferative in HeLa Cells

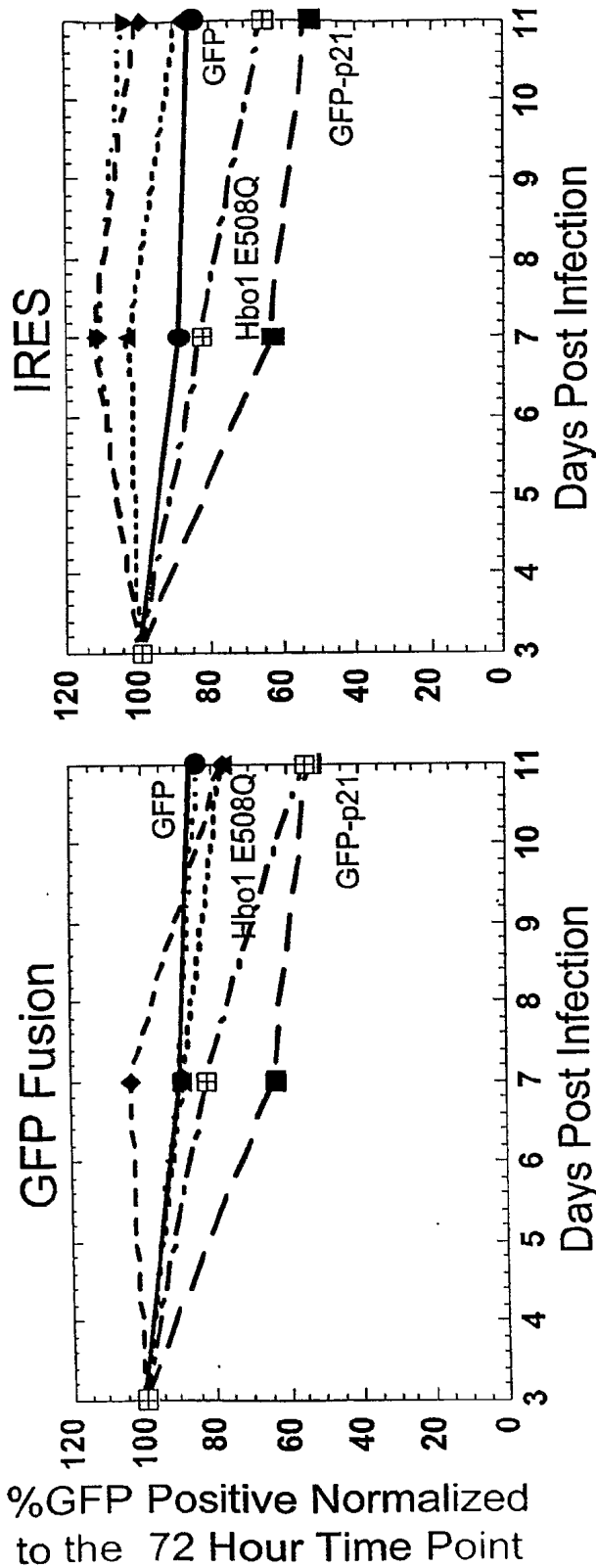
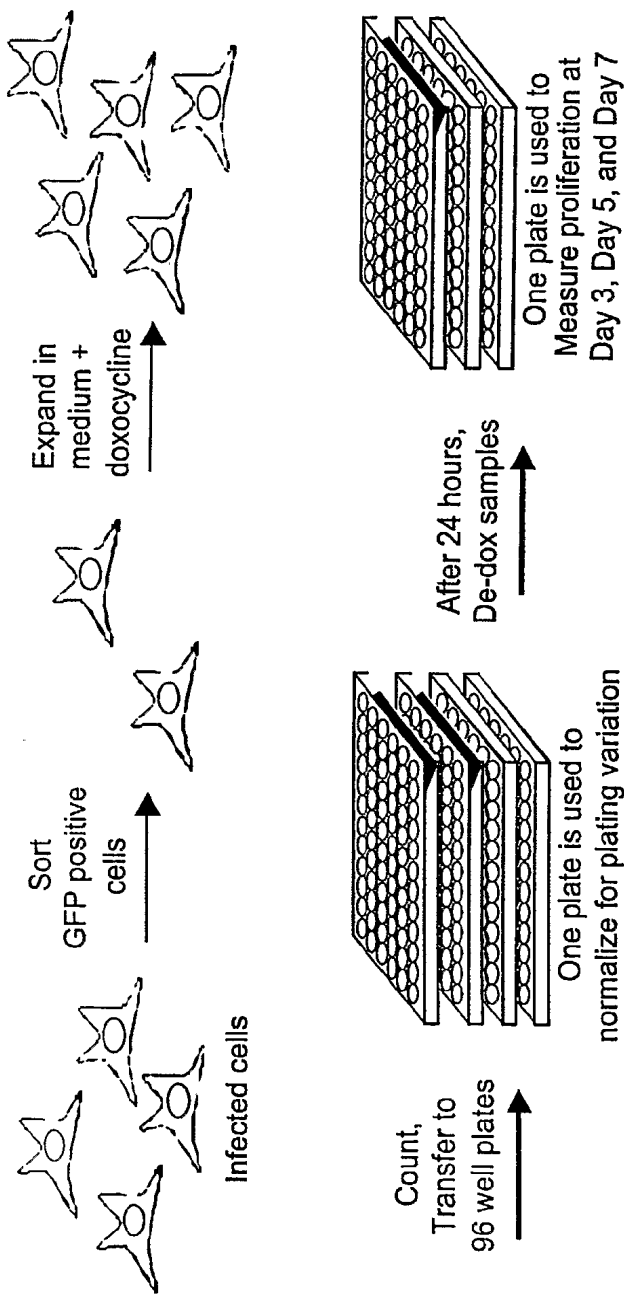


FIG. 40

Analyzing Proliferation of Sorted Cells Expressing HBO1 WT or Dominant Negative Mutants



Proliferation is measured using the CyQuant Cell Proliferation Assay (Molecular Probes) which is based upon the fluorescence enhancement upon binding of a proprietary dye to cellular DNA

FIG. 41

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Hbo1 E508Q is Antiproliferative in A549 Cells

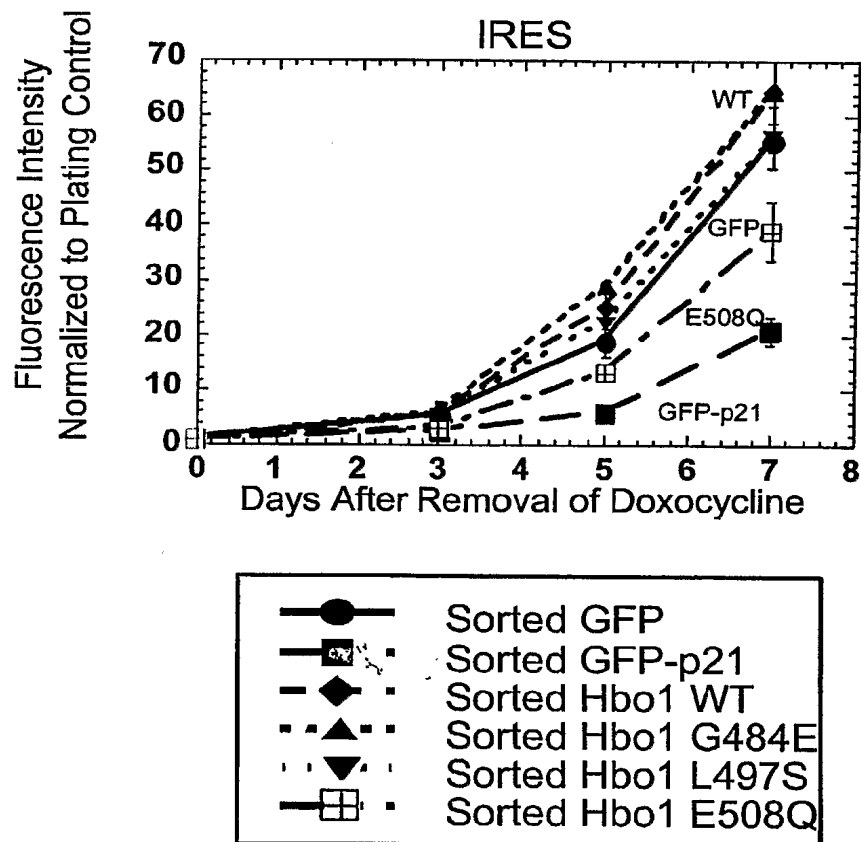
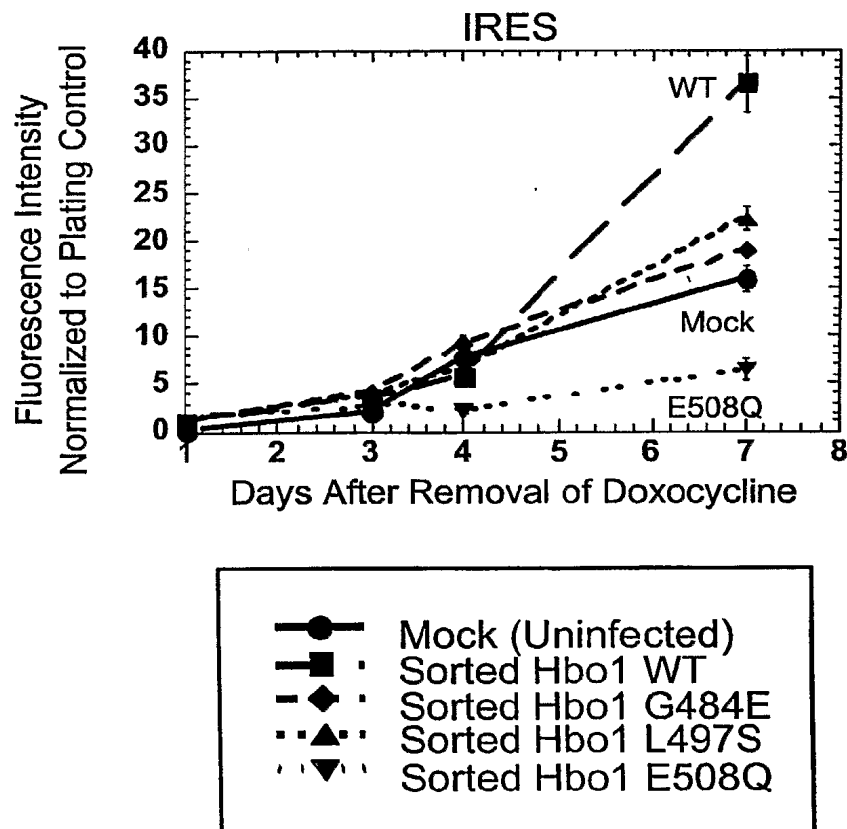


FIG. 42

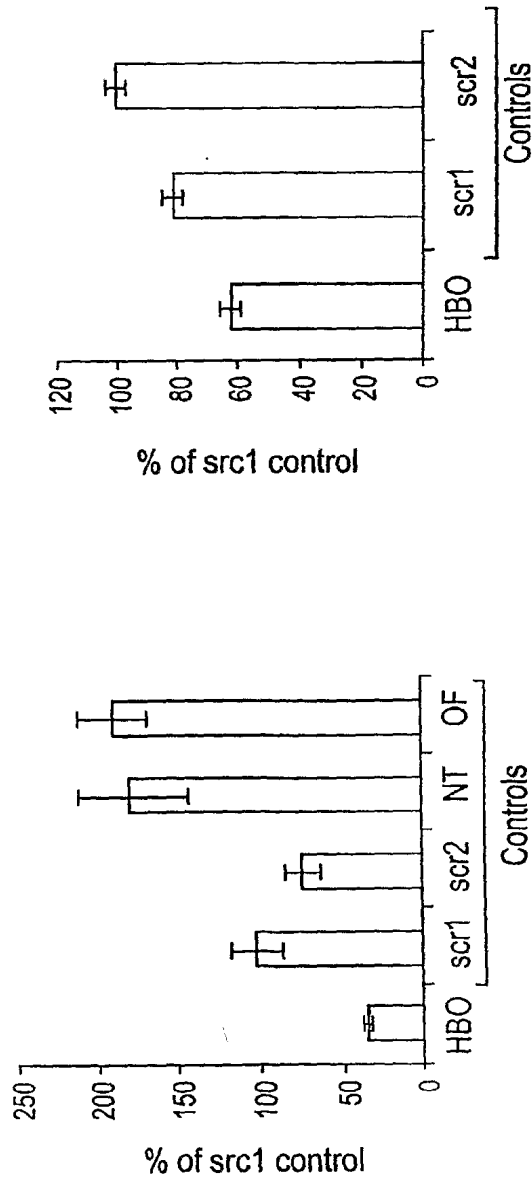
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Hbo1 E508Q is Antiproliferative in HeLa Cells



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HBO1-Specific siRNA Has an Antiproliferative Effect on A549 Cells



BrdU incorporation by A549 treated with HBO1 siRNA

HBO1 mRNA level in A549 after siRNA treatment (Taqman)

FIG. 44

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HBO1-Specific siRNA Has an Antiproliferative Effect on H1299 Cells

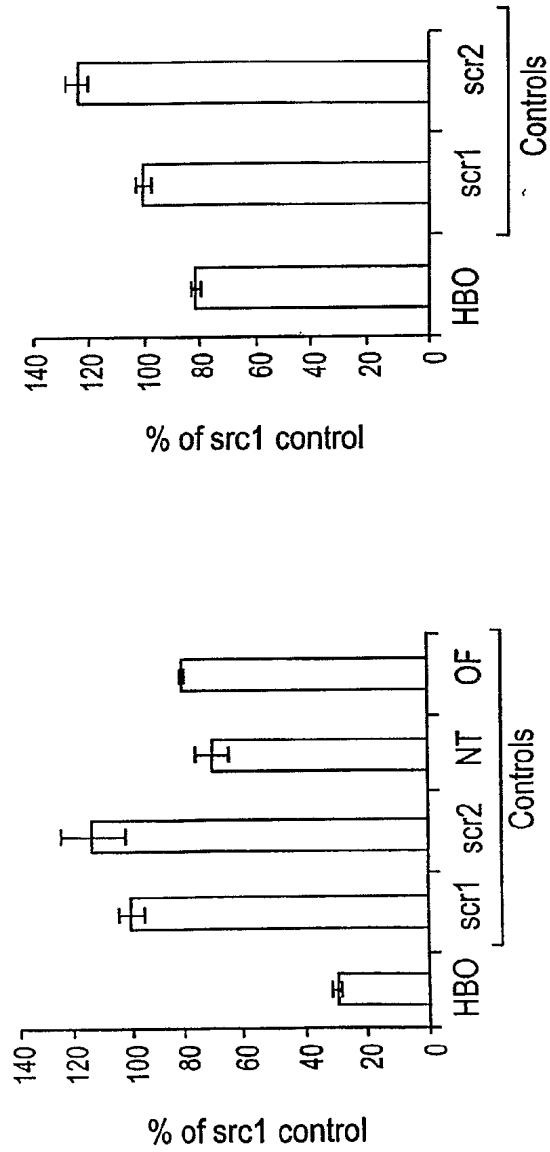
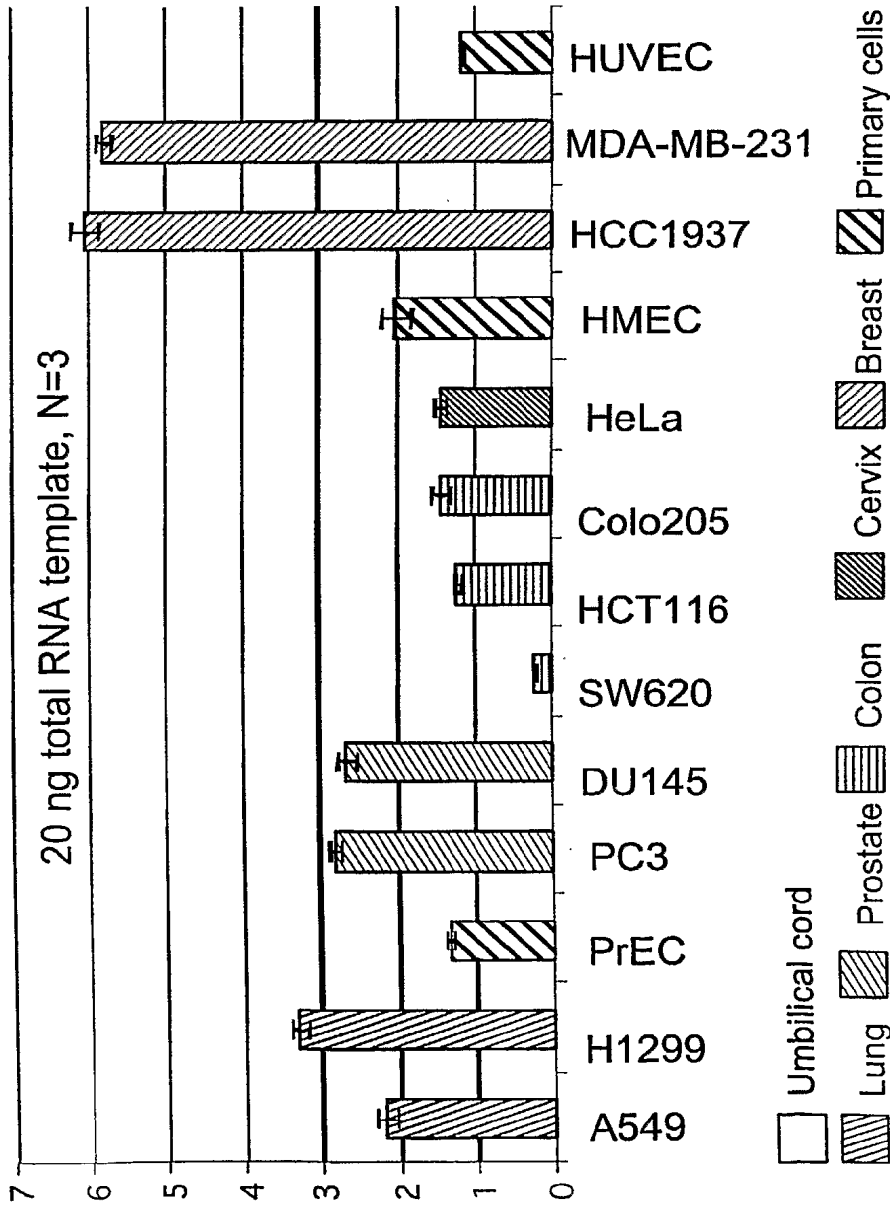


FIG. 45

FIG. 46

Taqman Analysis of PIM-1 Expression Using RNA from
Tumor Cell Lines



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Taqman Analysis of PIM-1 mRNA Expression
in Samples Obtained from Patients with Breast Carcinoma

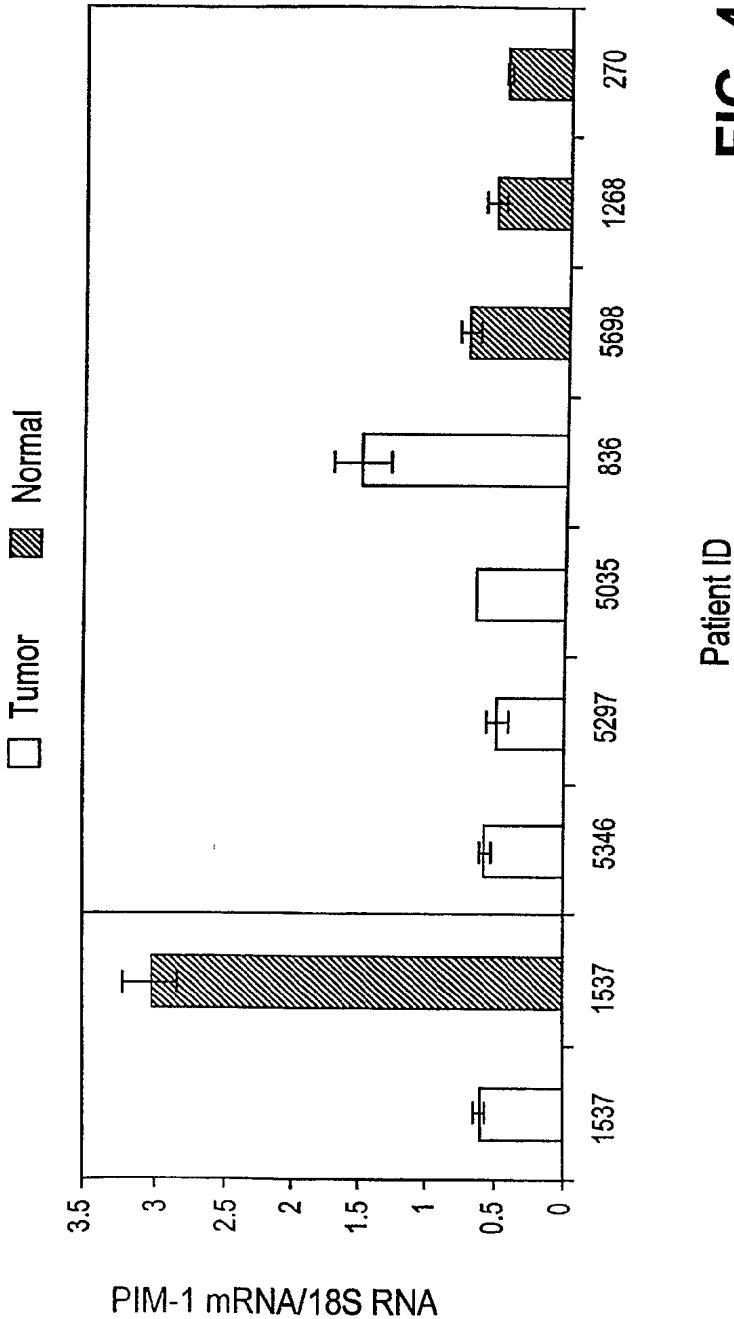


FIG. 47

N = 3, 20 ng total RNA/sample

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Taqman Analysis of PIM-1 mRNA Expression
in Samples Obtained from Patients with Lung Carcinoma

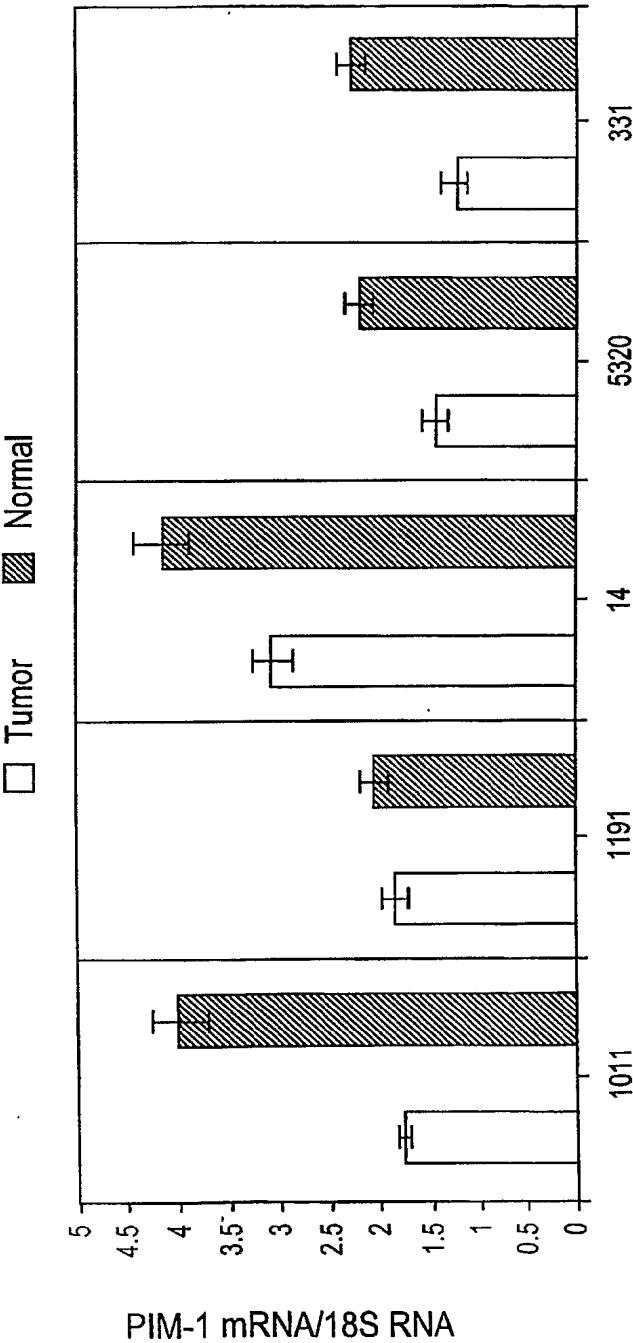


FIG. 48

Patient ID

N = 3, 20 ng total RNA/sample

Expression of Wild Type Pim-1 but not Dominant Negative Mutants
is Antiproliferative in A549 Cells

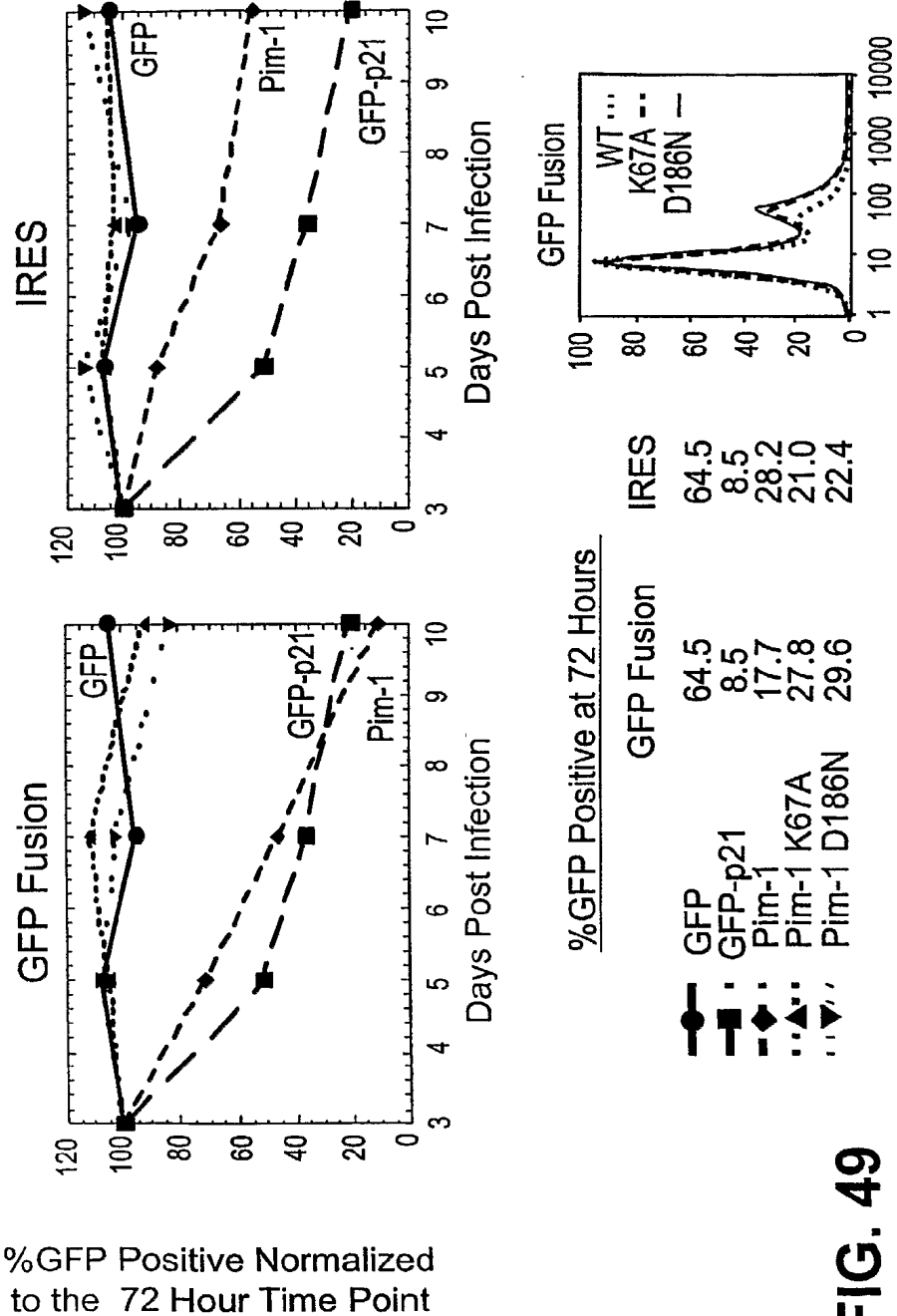
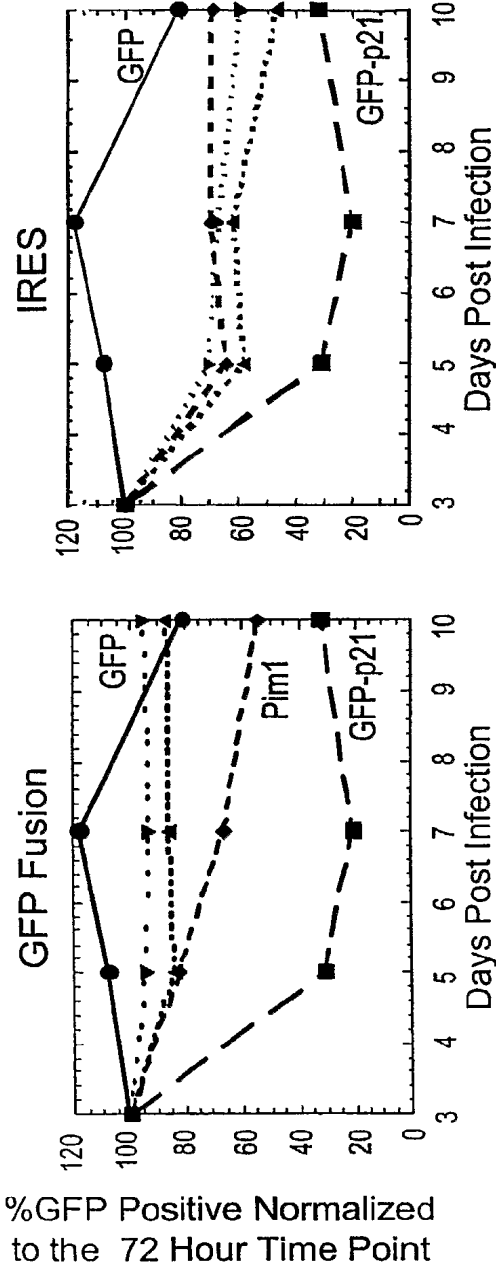


FIG. 49

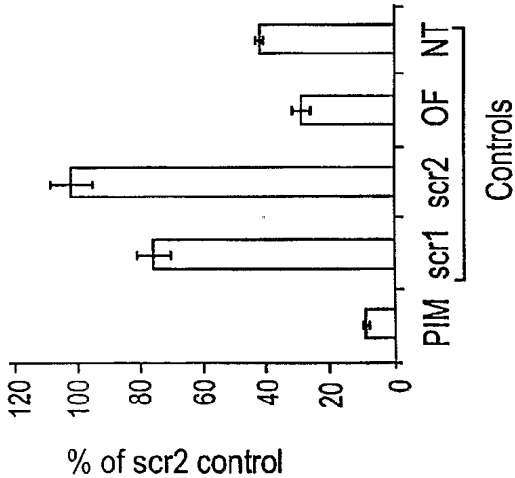
In H1299 Cells, Expression of GFP-Pim-1 WT and all IRES
Constructs is Antiproliferative



%GFP Positive at 72 Hours	
GFP Fusion	IRES
7.5	7.5
0.44	0.44
3.6	0.92
2.8	0.81
2.6	1.1

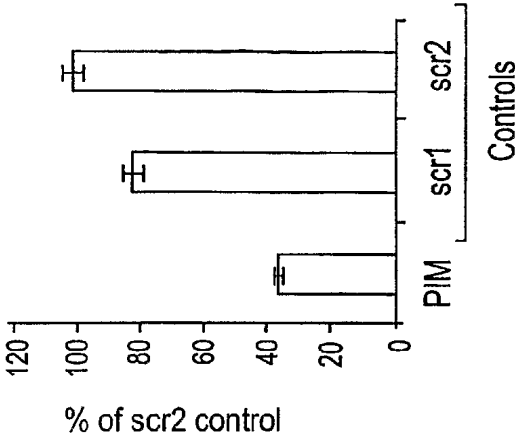
FIG. 50

PIM-1 Specific siRNA Has an Antiproliferative Effect on A549 Cells



PIM-1 mRNA level in A549 after siRNA treatment (Taqman)

OF: oligofectamine, NT: no transfection

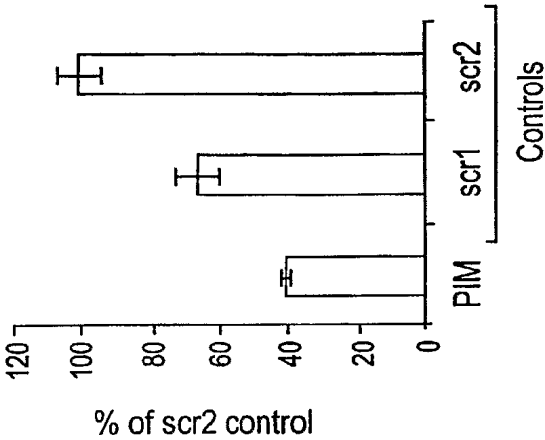


BrdU incorporation by A549 treated with PIM-1 siRNA

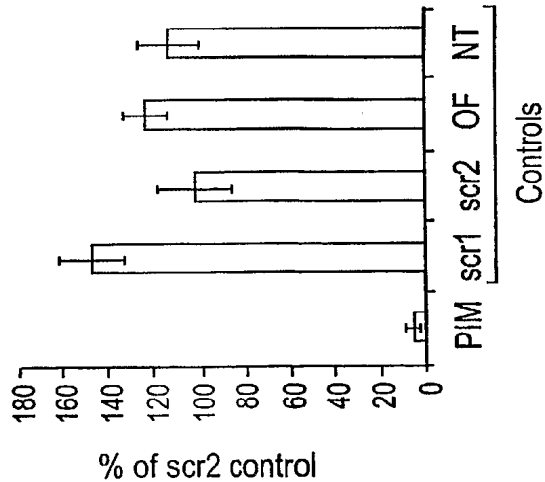
FIG. 51

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PIM-1 Specific siRNA Has an Antiproliferative Effect on HeLa Cells



BrdU incorporation by HeLa treated with PIM-1 siRNA

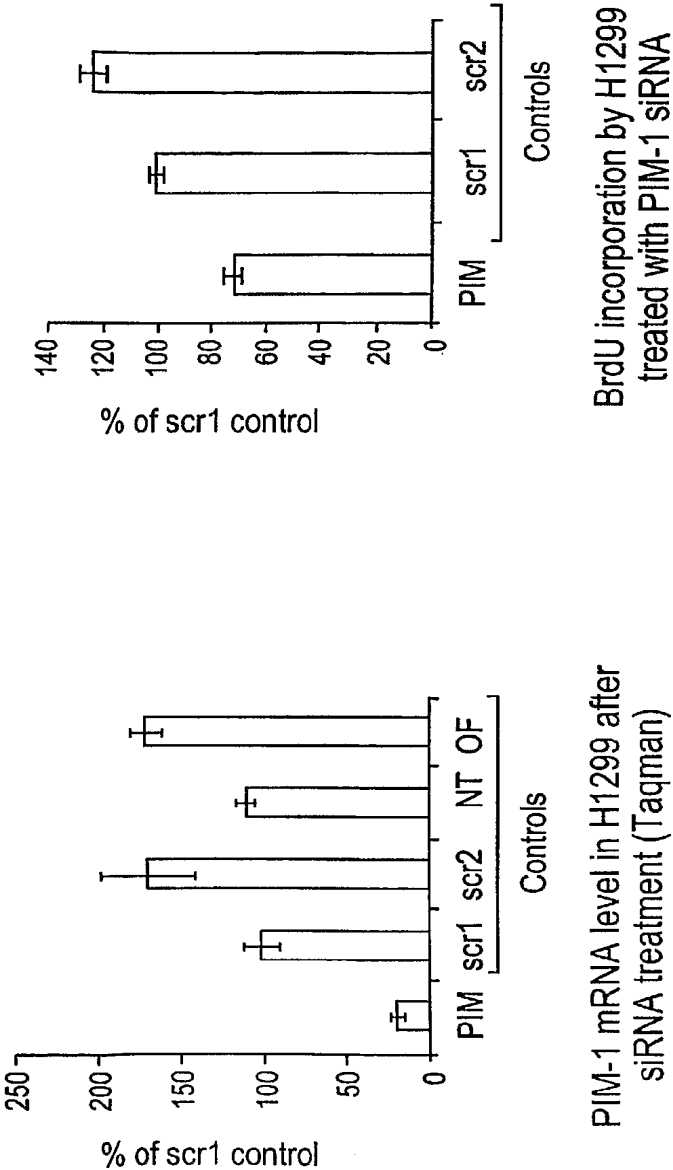


PIM-1 mRNA level in HeLa after siRNA treatment (Tagman)

OF: oligofectamine, NT: no transfection

FIG. 52

PIM-1 Specific siRNA Has an Antiproliferative Effect on H1299 Cells

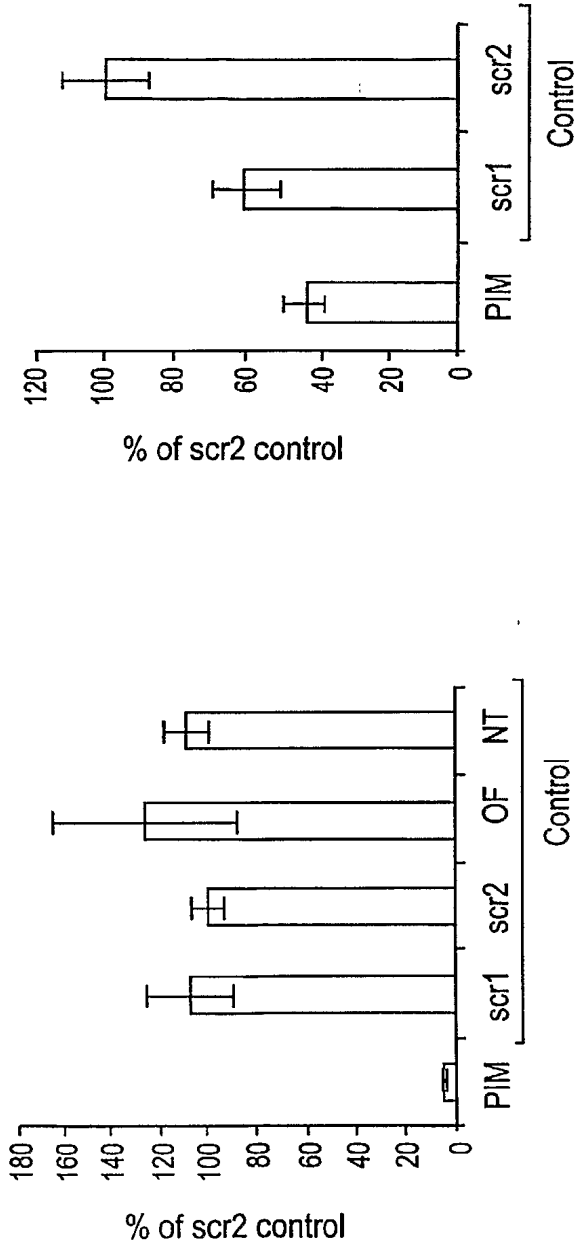


OF: oligofectamine, NT: no transfection

FIG. 53

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PIM-1 Specific siRNA Has an Antiproliferative Effect on HUVEC Cells



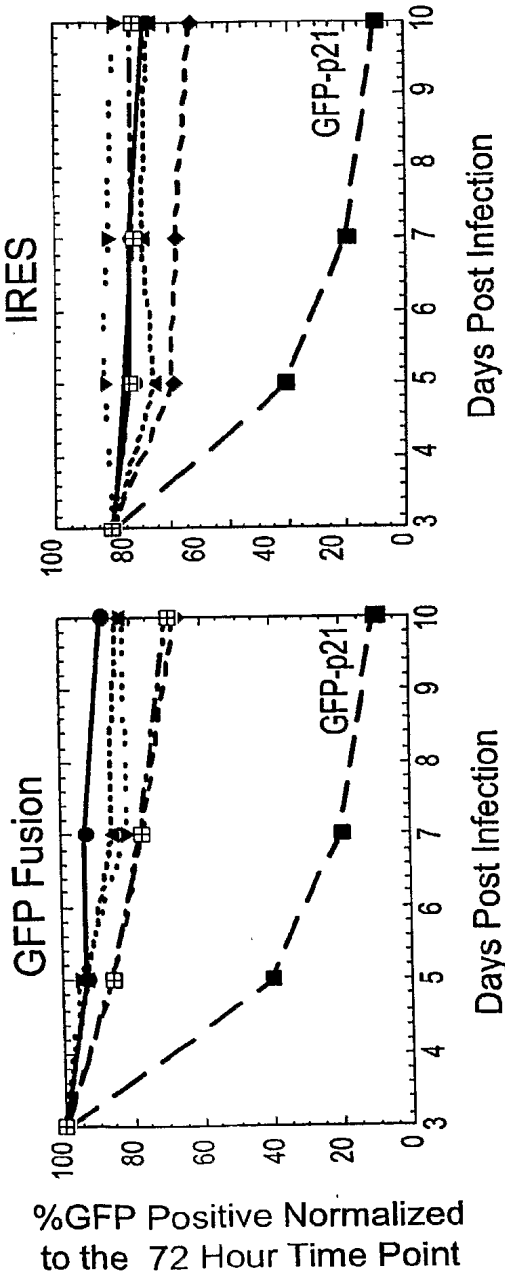
BrdU incorporation by HUVEC treated with PIM-1 siRNA

PIM-1 mRNA level in HUVEC after siRNA treatment (Tagman)

OF: oligofectamine, NT: no transfection

FIG. 54

No Antiproliferative Effects are Observed for Ape1 WT and
Dominant Negative Mutants in A549 Cells



%GFP Positive at 72 Hours		
	GFP Fusion	IRES
GFP	66.4	66.4
GFP-p21	13.2	13.2
Ape1	13.2	13.6
Ape1 E96A	12.9	16.7
Ape1 D210A	14.3	11.3
Ape1 C65A	14.6	17.2

FIG. 55

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GFP-Ape1 WT and Dominant Negative Mutants Have No Effect in H1299 Cells

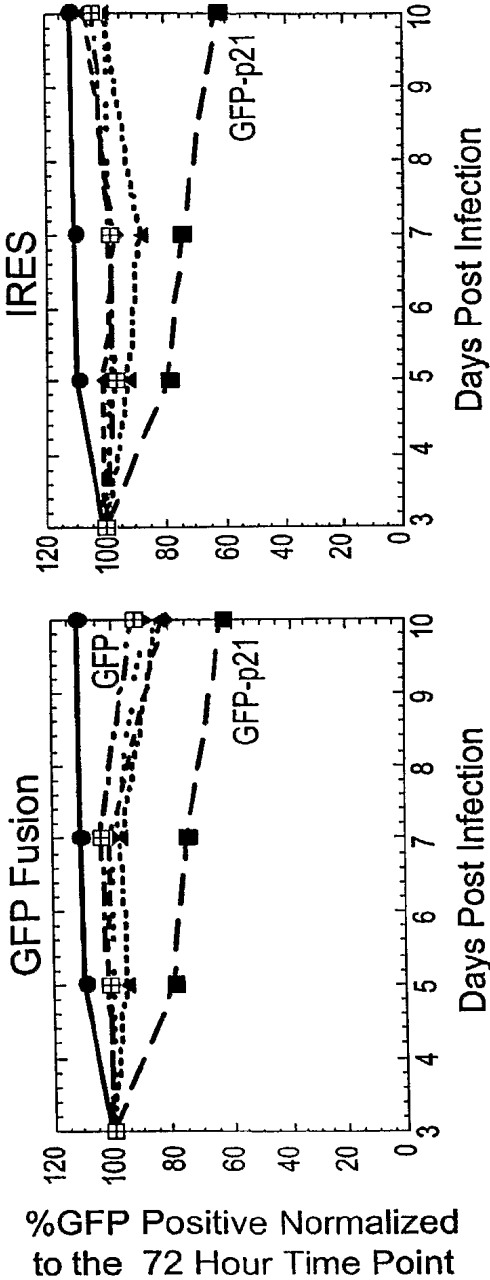
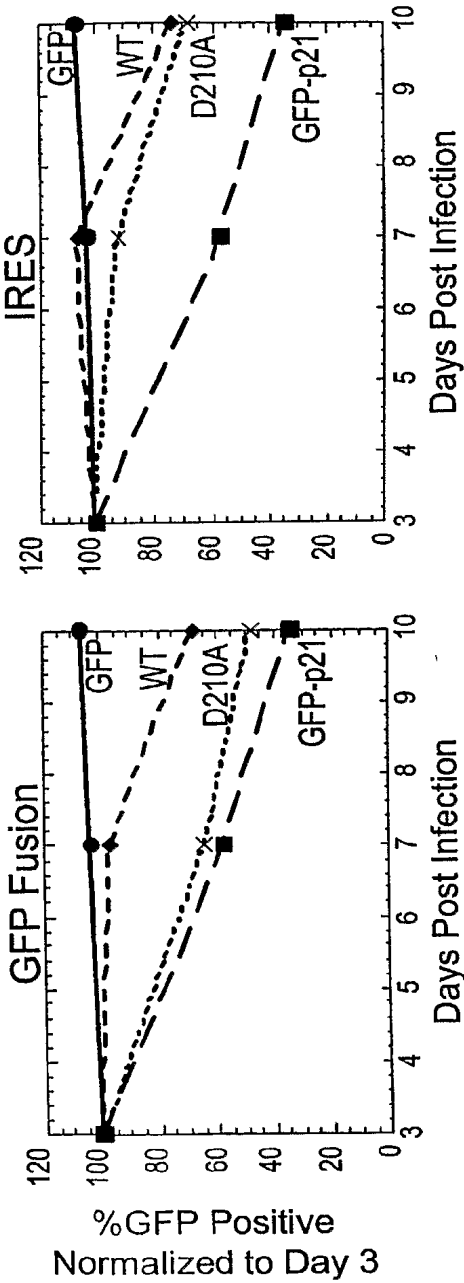


FIG. 56

Both Ape1 WT and Ape1 D210A Are Antiproliferative in HMEC Cells

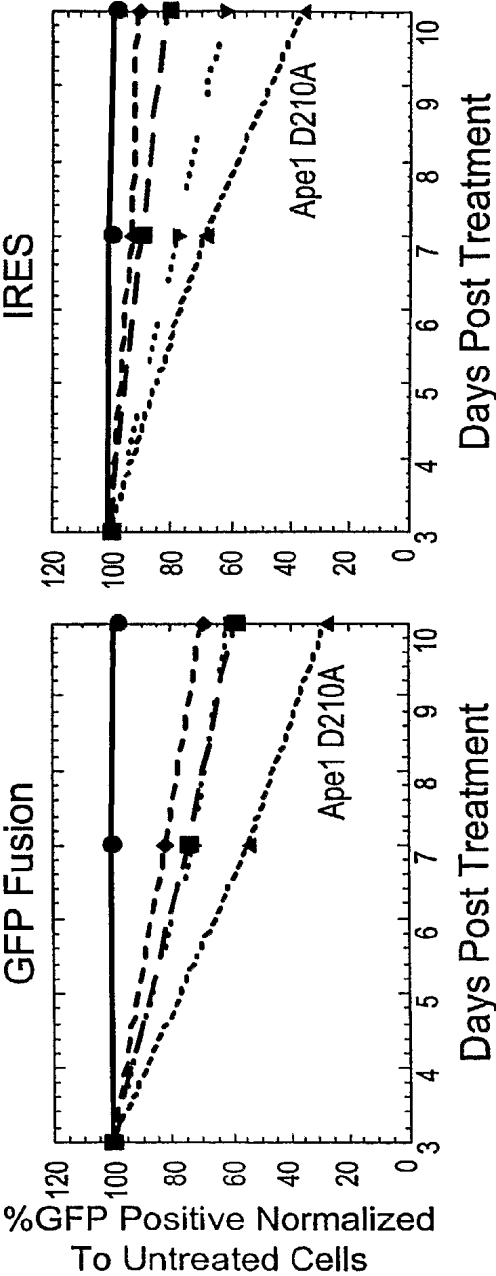


%GFP Positive (Day 3)

	GFP	IRES
GFP	30.7	30.7
GFP-p21	5.6	5.6
Ape1 WT	3.6	4.8
Ape1 D210A	5.2	4.2

FIG. 57

Ape1 D210A Sensitizes A549 Cells to Methyl Methanesulfonate Treatment



At 72 hours after infection, A549 cells were treated with 3mM MMS for 60 min.

	GFP Fusion	IRES
GFP	64.8	64.8
Ape1	15.7	6.2
Ape1 E96A	17.2	9.9
Ape1 D210A	11.3	6.1
Ape1 C65A	16.8	3.3

FIG. 58

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Ape1 WT and C65A Are Protective in A549 Cells Treated with Bleomycin

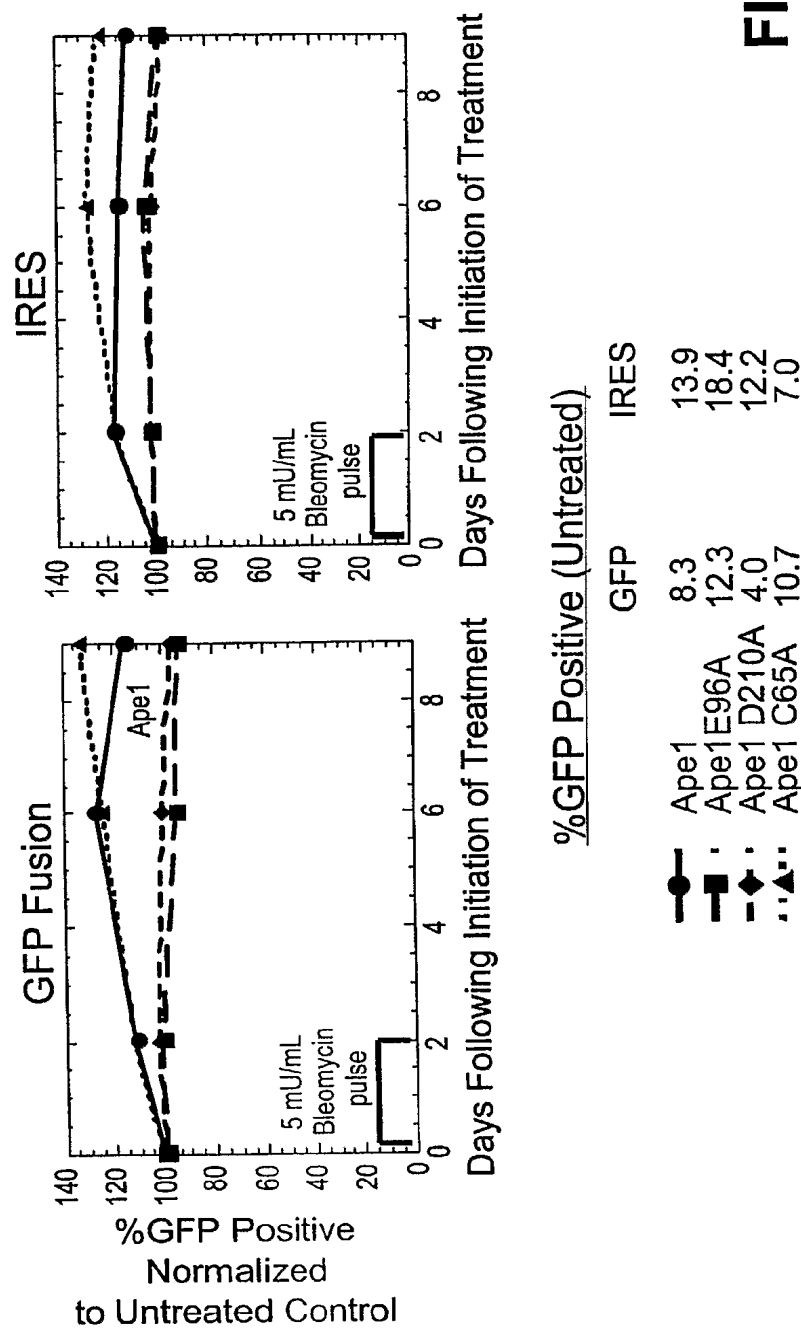
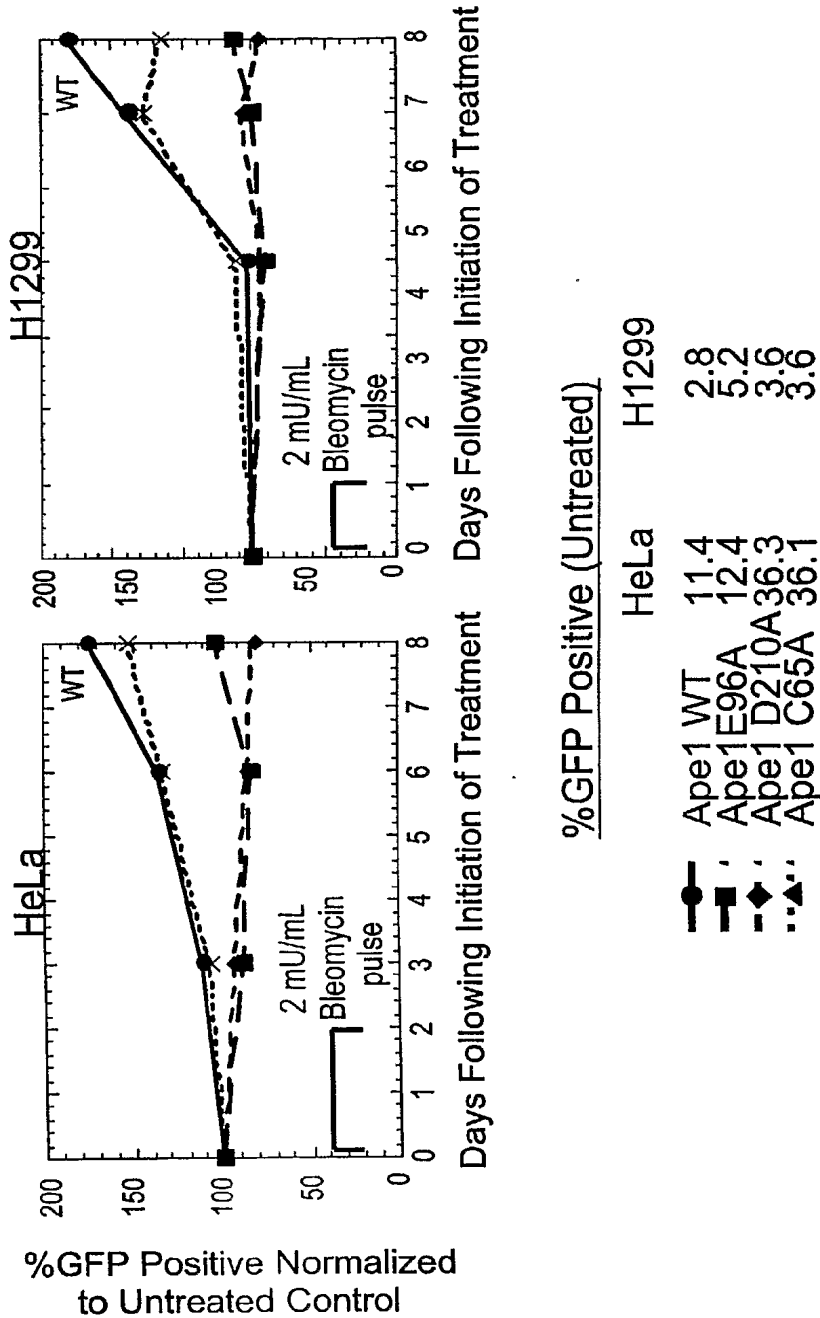


FIG. 59

These results are consistent with those published by Robertson et al., *Cancer Res.* 2001 showing that overexpression of Ape1 in the tumor line NT2 confers resistance to bleomycin treatment.

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Ape1 WT and C65A Are Protective in HeLa and H1299 Cells
Treated with Bleomycin



IRES-Ape1 constructs were used for these experiments.

FIG. 60

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Tagman Analysis of CK2 α Expression Using RNA from
Tumor Cell Lines

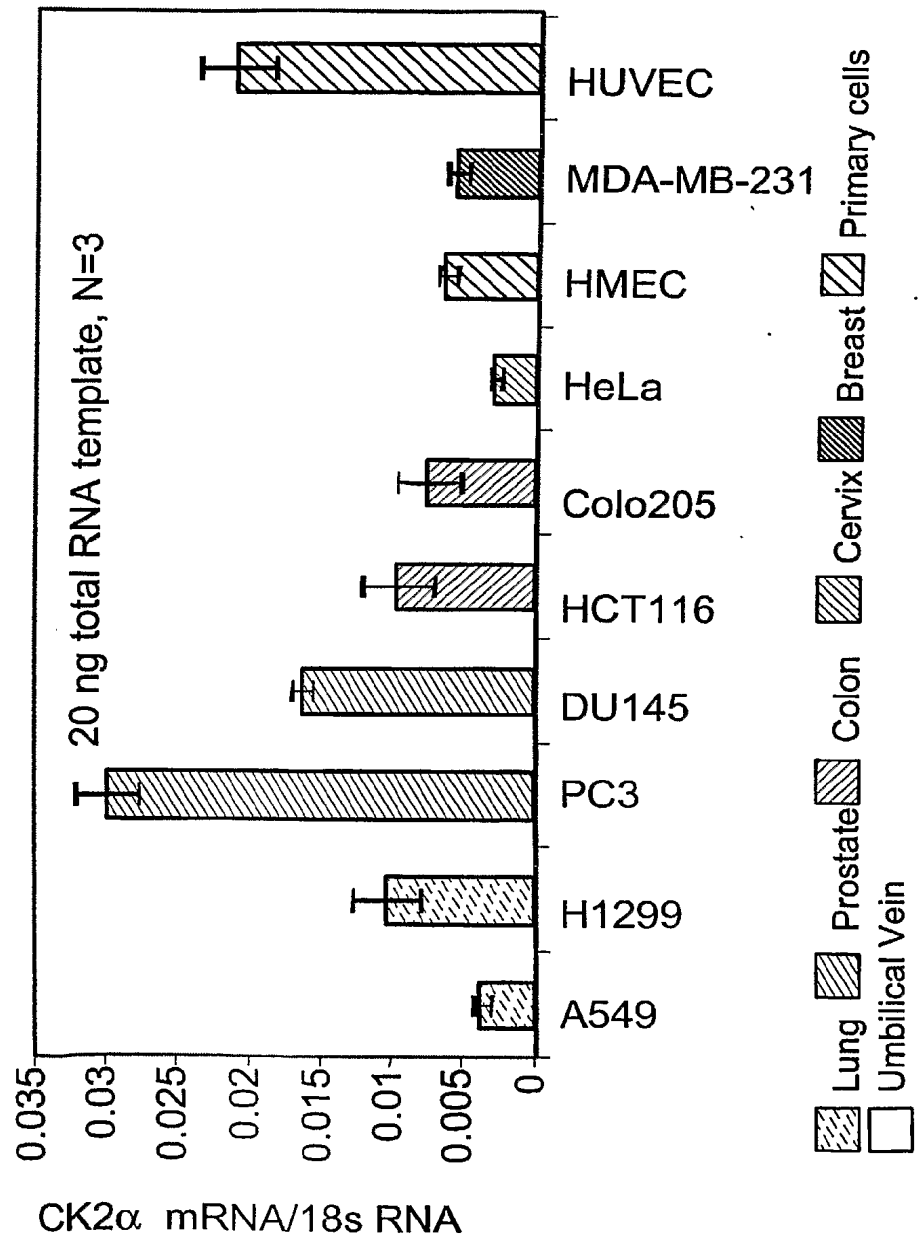


FIG. 61

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Dominant Negative Mutants for CK2_

Point mutants : K68A, D175N- K68A corresponds to a mutation considered essential for the phosphotransfer reaction in the kinase domain (Oncogene. 2001 Apr 12;20(16):2010-22. PMID: 11360185). D175N is a mutation in the activation loop of the kinase domain. (Mol Gen Genet. 1997 May 20;254(5):562-70.PMID: 9197416)

```

*->yelleklGeGsfGkVykhkdkgtgkiVAvKilkkkesikekrflr
    y+l++klG+G +++V++a+++  ++++V+vKilk  ++k+  + r
CK2alpha 39  YQLVRKLGRKYSEVFEAINI-TNNEKVVVKILK--PVKKKK--IKR 80

    EiqilkrLs.HpNIvrligvfed.tddhlylvmEymegGdLfdylrrngg
    Ei+il +L++ pNI++l +++ d+ ++ + lv+E++++d +++ + +
CK2alpha 81  EIKILENLRGPNIIITLADIVKDpVSRTPALVFEHVNNNTDFKQLYQTLT- 129

    plsekeakkialQilrGleYlHsngivHRDLKpeNILLdendgtvKiaDF
    + +++++++il++l+Y+Hs+gi+HRD+Kp N++++d+++ +++++D+
CK2alpha 130 ---DYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHRKRLRLDW 176

    GLArlesssklttfvGtpwYmmAPEvileg.rgysskvDvWSlGvilyE
    GLA ++++++ ++ +v ++++ PE+ l + ++y+ D+WSlG++L+
CK2alpha 177 GLAEFYHPGQYNNVRVASRYFK-GPEL-LVDyQMYDYSldMWSlGCMlAS 224

    lltggplfpgadlpafg.gd.evdqli.if.vlklPfsdelpktridpl
    + +++++f+  ++++++++ ++ ++++ +d+++k++i+
CK2alpha 225 MIFRKEPFF-----HgHDnYDQLVLRiAKvLGTEdLYDYIDKYNIeLD 266

    eelfriikrp.....glrlplpsncSeelkdLlkkcInkDpskRpGsa
    + +i+ r+++++ +++ ++++++++S+e++d+l+k+l++D ++R+
CK2alpha 267 PRFNdILGRHsrkrwerFVHSENQHLVSPEALdFLDKLLRYDHQSRL--- 313

    takeilnhpwf<-*
    ta+e++hp+f
CK2alpha 314 TAREAMEHPYF 324 Bold = the catalytic residues

```

FIG. 62

CK2_ -Specific siRNA is Antiproliferative in H1299 Cells

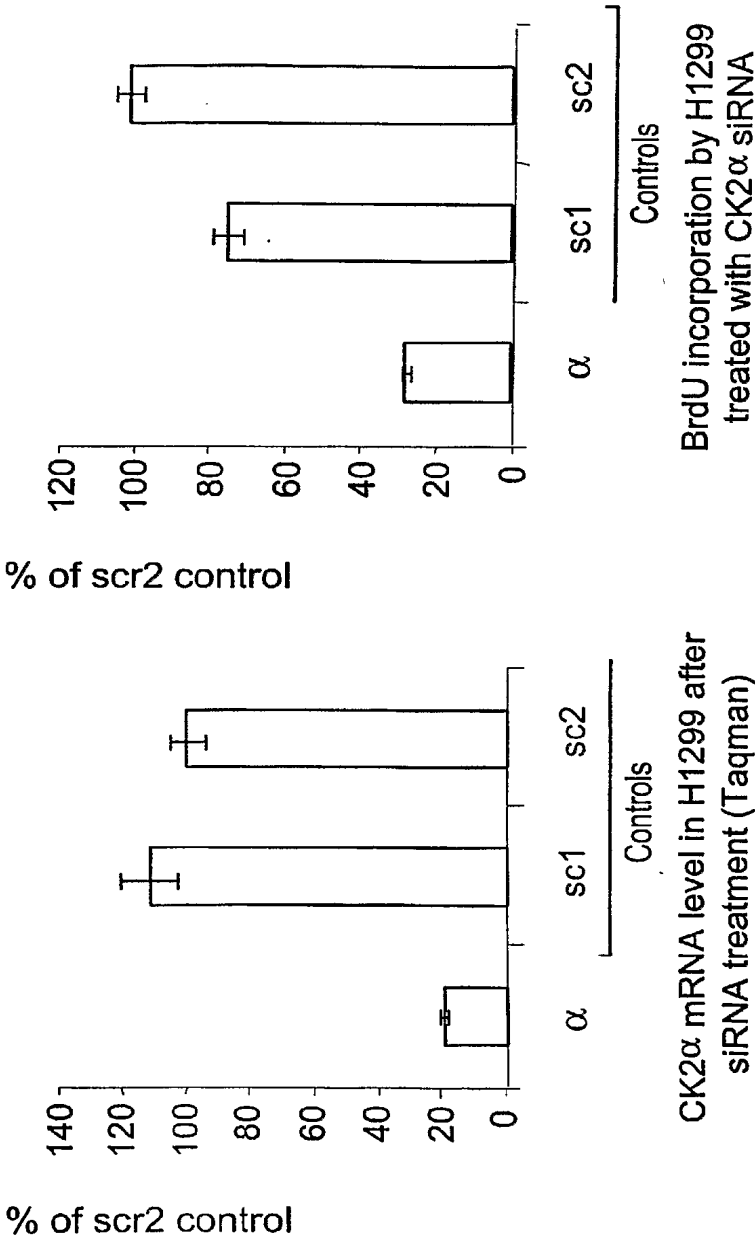


FIG. 63

Sc1 and sc2 refer to scrambled siRNA controls

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Taqman Analysis of NKIAMRE Expression Using RNA from
Tumor Cell Lines

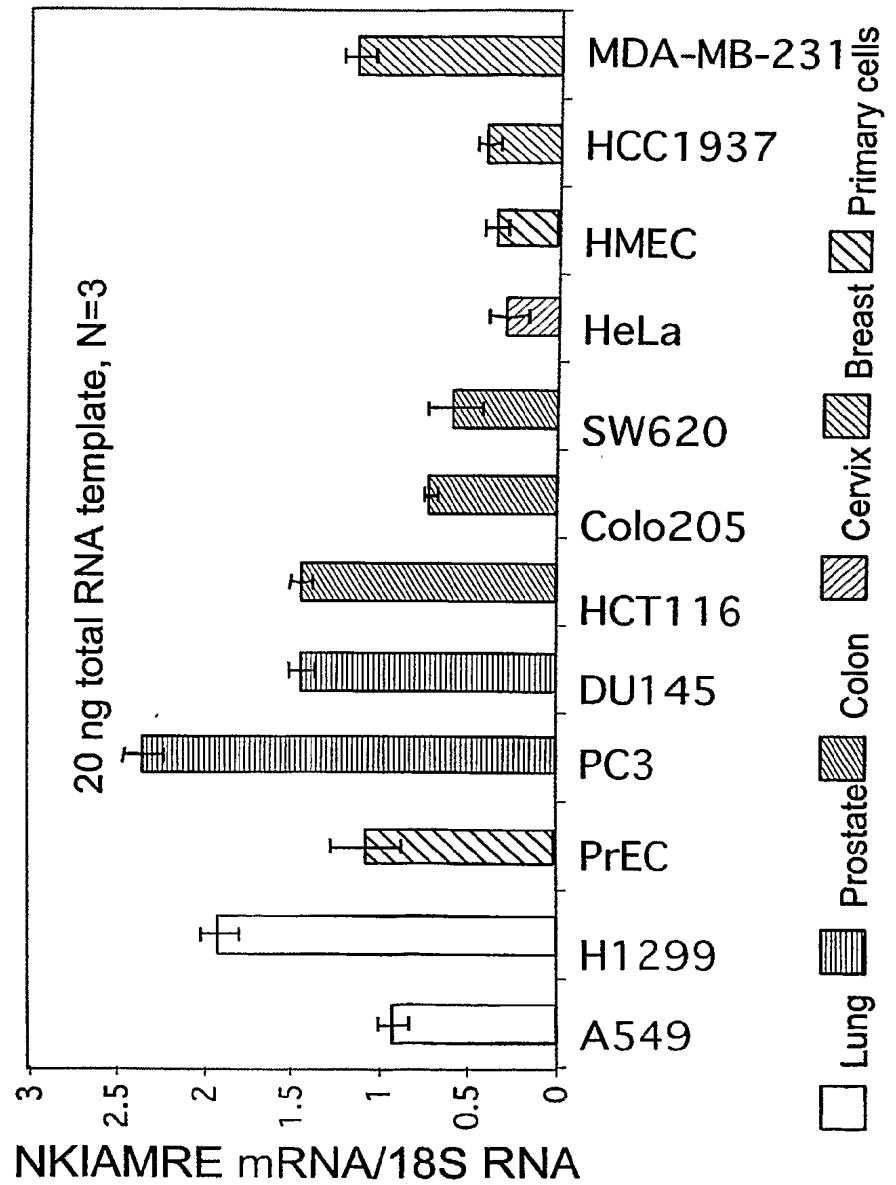


FIG. 64

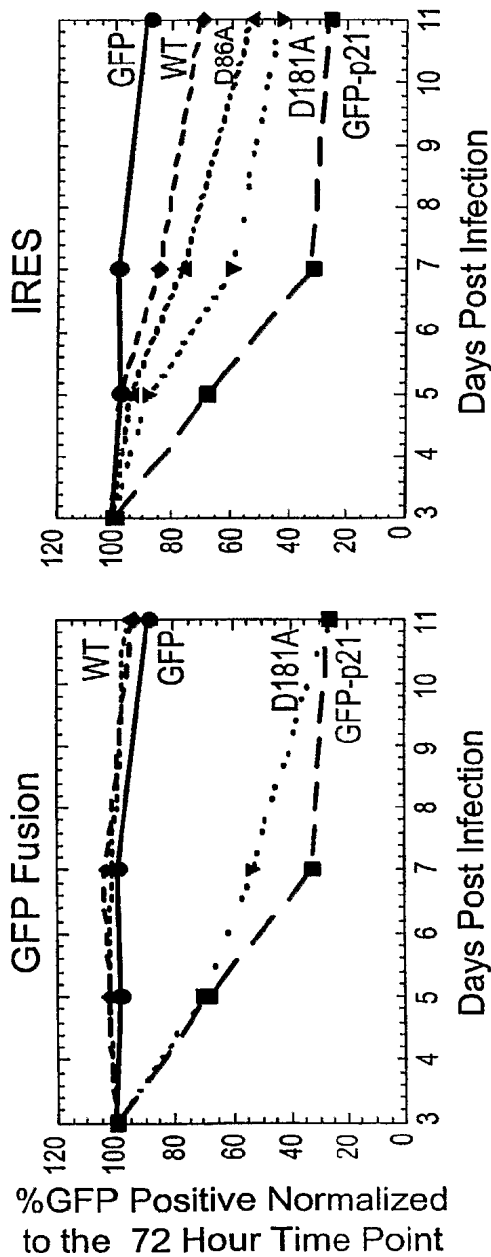
Dominant Negative Mutants for FEN1

XPG_N domain*->	MGIkGLlpiLkpvapeairsvsiEalegYYkvLAiDasilwLyqfLka	
	MGI+GL+++++vap+air++++i++++g +++AiDas++++yqfL+a	
FEN1 1	MGIQGLAKLIADVAPSAIRENDIKSYFG--RKVAIDASMSIYQFLIA 45	
		○
XPG_N domain	vRdqlgnnlenEeGettshlmglfsRlcrLldfgIkPiFVFDGgapndLK	
	vR q g+ l+nEeGettshlmg+f+R+++++gIkP++VFDG++p +LK	
FEN1 46	VR-QGGDVLQNEEGETTSHLMGMFYRTIRMENGIKPVYVFDGKPP-QLK 93	
		○
XPG_N domain	aetlqKRsarrrqea<-*	
	+++l+KRs+rr+tea	
FEN1 94	SGELAKRSEERRAEA 107	
		○
XPG_I domain*->	rlmGIpyIvAPgVEAEAQcayLekkglvdgiiteDsDvLLFGaprll	
	+lmGIpy +AP+ EAEA ca+L+k+g+v++++TeD+D+L FG+p+l+	
FEN1 146	SIMGIPYLDAPS-EAEASCAALVKAGKVYAAATEDMDCLTFGSPVLM 191	
		○
XPG_I domain	rnltlsgkksgPsitslkveieeidesllreLgIgklsreqLidlaiLl	
	r+lt s++k k++i+e++l+++l+elgL ++eq++dl+iIl	
FEN1 192	RHLTASEAK-----KLPIQEFHLRILQELGL---NQEQFVDLCILL 230	
		○
XPG_I domain	GcDYteG<-*	
	G+DY+e+	
FEN1 231	GSDYCES 237	
		○ Mutation site

FIG. 66

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Expression of FEN1 Dominant Negative Mutants in A549 Cells
is Antiproliferative



%GFP Positive at 72 Hours

	GFP Fusion	IRES
GFP	85.3	85.3
GFP-p21	19.5	19.5
Fen1 WT	65.0	60.1
Fen1 D86A	68.0	58.0
Fen1 D181A	48.3	59.7

FIG. 67

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Expression of FEN1 Dominant Negative Mutants is Antiproliferative in H1299 Cells

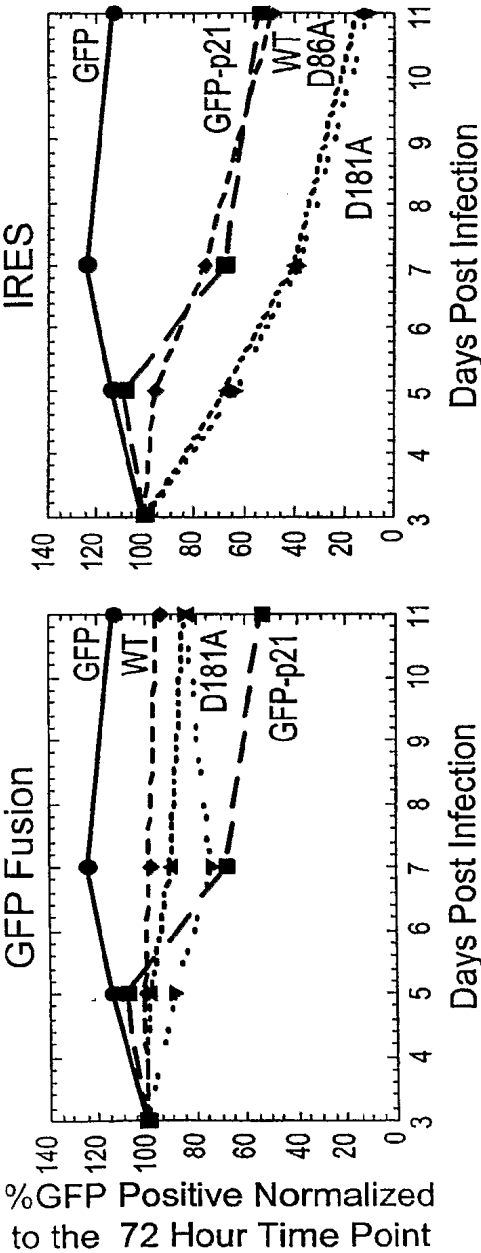
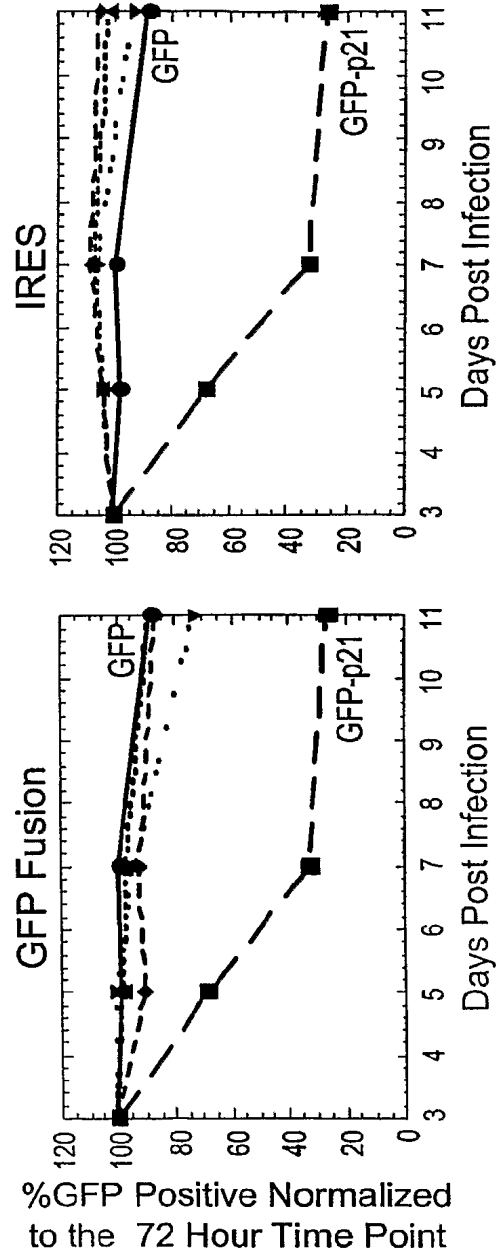


FIG. 68

%GFP Positive at 72 Hours		GFP Fusion	IRES
●	GFP	58.1	58.1
■	GFP-p21	3.9	3.9
◆	Fen1 WT	34.4	25.9
▲	Fen1 D86A	34.1	58.0
▼	Fen1 D181A	48.3	59.7

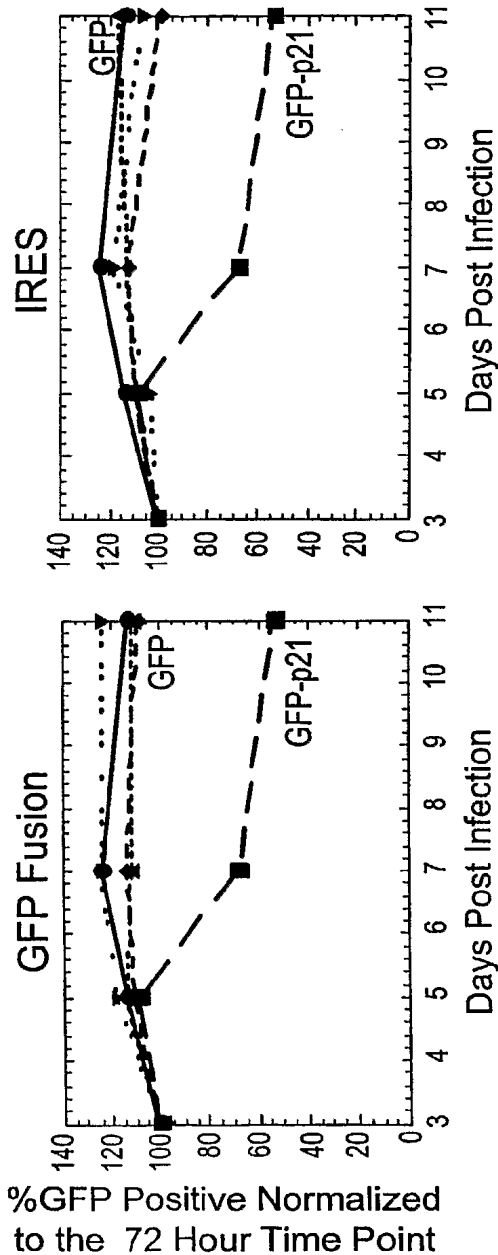
Expression of CDK3 Dominant Negative Mutants Has No
Antiproliferative Effect in A549 Cells



%GFP Positive at 72 Hours		
GFP Fusion	IRES	
85.3	85.3	GFP
19.5	19.5	GFP-p21
41.3	57.2	CDK3 WT
41.9	57.7	CDK3 K33A
34.6	51.5	CDK3 D145A

FIG. 70

Expression of CDK3 Dominant Negative Mutants Has No Antiproliferative Effect in H1299 Cells



%GFP Positive at 72 Hours

	GFP Fusion	IRES
● GFP	58.1	58.1
■ GFP-p21	3.9	3.9
◆ CDK3 WT	24.8	30.0
▲ CDK3 K33A	27.0	32.2
▼ CDK3 D145A	21.7	28.7

FIG. 71

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Dominant Negative Mutants for HBO1

HBO1	YHSPYPEEYARLGRLYMCEFLKYMKSQTILRRHMAKCVWKHPDGGDEIYRKGSISVFEVD	
yEsa1	YFSPYPIELTDEDFIYIDDFTLQYFGSKKQYERYRKKCTLRHPPGNEIYRDDYVSFFEID	
	*.*** * : . : * : * : * : * : * : * : * : * : * : * : * : * : *	
HBO1	GKKNKIYCQNLCLLAKLFLDHKTLYYDVEPFLFYVMTEADNTGCHLIGYFSKEKNSFLNY	
yEsa1	GRKQRTWCRLNCLLSKLFLDHKTLYYDVPFLFYCMTRRDELGHHLVGYSKEKESADGY	
	*.*** : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
HBO1	NVSCILTMPQYMRQGYGKMLIDFSYLLSKVEEKVGSPERPLSDGLISYRSYWKVLLRY	
yEsa1	NVACILTLPOYQRMGYGKLLIEFSYELSKKENKVGSPKPLSDGLLSYRAYWSDTLITL	
	*.*** : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
HBO1	LHNFQGEISIKEISQETAVNPVDIVSTLQALQMLKYWKGHVLKRQDLIDEWIAKEAK	
yEsa1	LVEHQ-KEITIDEISSMTSMTTDLHTAKTLNLRYYKQGHIIFLNEDILDYRNRLKAK	
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
HBO1	RSNSNKTMDPSCCLKWTPPKGT-----	
yEsa1	K---RRTIDPNRLIWKPPPVFTASQLRFAW	
	: : : : * : * : * : *	

○ Mutation site

FIG. 72

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Dominant Negative Mutants for PIM-1

*->yellekl**Ge**GsfGkVykakhkdkdtgkiVAv**K**ilkkekesikek....
 y+++ l**G**+**G**+fG+Vy ++++ +++ +VA+**K** + k +i+++++ +
 PIM1 38 YQVGPLL**GS**GGFGSVYSGIRV-SDNLPVA**IK**HVE--KDRISDWgelp 81

rflrEiqilkrLs..HpNIvrligvfedtdddhlylvmeYmegG.dLf
 +++r+ +E+ +lk++s++ ++rl+++fe ++d++ l++E e +dLf
 PIM1 82 ngtrVPMEVVLLKKVSsgFSGVIRLLDWFE-RPDSFVLILERPEPVqDLF 130

dylrrnggplsekeakkialQilrGleYLHsngivHRDL**K**pe**NIL**ldend
 d++++g +l e+ a+++++Q+l+++ ++H++g++HRD+**K** e**NIL**+d n+
 PIM1 131 DFITERG-ALQEELARSFFWQVLEAVRHCHNCGVLHRD**IK**DENILIDLNR 179

gtvKia**D**FGLArlllesssklttfvGTpwYmmAPEvileg.rgysskvDvW
 g +K++**D**FG +ll+ ++ +t+f GT++Y +PE+ ++++r++++ + vW
 PIM1 180 GELKL**D**FGSGALLK-DTVYTDFDGT RVYS-PPEW-IRYhRYHGRSAAVW 226

SlGviLyElltggplfp gadlpaf tggdevdqliifvklPfsdelpktr
 SlG++Ly +++g ++Pf++
 PIM1 227 SLGILLYDMVCG-----DIPFEH----- 244

idpleelfriikrpglrlplpsncSeelkdLlkkcLnkDPskRpGsatak
 ee+ r++ + +++S+e+++L+++cL++ Ps+Rp t++
 PIM1 245 ---DEEIIRQVF-----FRQVSSECQHILRWCLALRPSDRP---TFE 282

eilnhpwf<-*
 ei nhpw+
 PIM1 283 EIQNHPWM 290

○ Mutation site

FIG. 73

No Significant Antiproliferative Effect is Observed with GFP-NKIAMRE
Dominant Negative Mutants in Either A549 or H1299 Cells

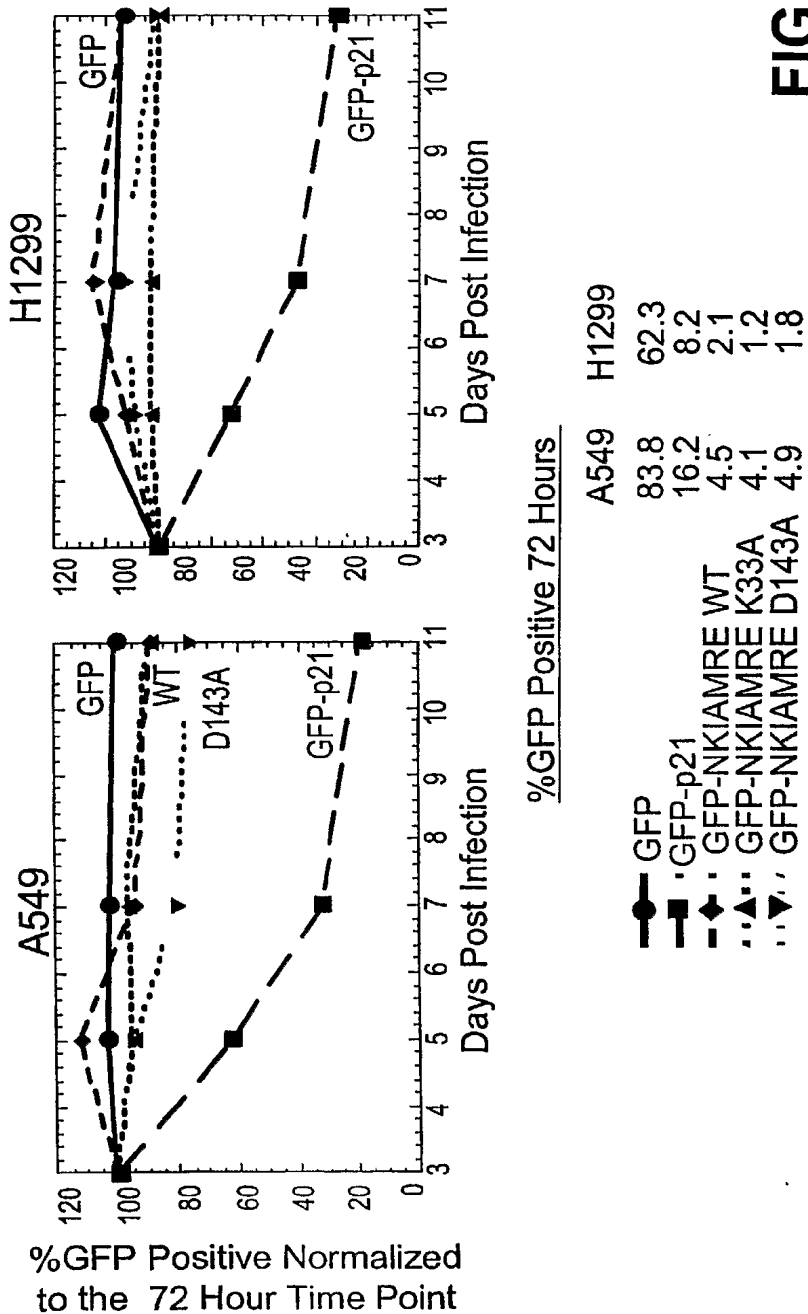


FIG. 74